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STUDIES ON ACQUIRED IMMUNITY WITH TOBACCO AND AUCUBA MOSAICS

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Most plants that became infected with virus diseases retain the etiological agents as long as they live. If they acquire immunity, it must be of the nonsterile type, such as is known for certain viroses of animals that do not show the usual sterile type of immunity.

Because of their wide host ranges and the ease with which they may be transmitted, plant virus diseases are well suited for studies on immunity. This is especially true of the systemic ones that do not kill plants, for they can be held under observation for long periods of time. Moreover, there is evidence that such diseases may be limited in their severity by some mechanism which prevents undue multiplication of the infective principle.

The occurrence in nature of closely related strains of certain virus diseases would seem to offer favorable possibilities for cross-immunization studies, since the strains frequently show considerable differences in severity. It would be desirable from both the practical and scientific viewpoints if it could be shown that plants infected with one strain are thereby protected against infection by other strains, including those of greater severity. Studies were, therefore, undertaken for the purpose of determining whether plants inoculated simultaneously with two viruses that are presumed to be related strains of tobacco mosaic will come down with both or with only one of the strains, and whether plants having either of the two strains can be infected with the other. The ordinary field type of tobacco mosaic and aucuba mosaic of tomato were used in the experiments to be reported in this paper.

LITERATURE

On Acquired Immunity in Plants. A review of the literature on acquired immunity in plants has recently been published by Chester (9). A few of the most important papers on acquired immunity from viroses of plants will be mentioned here.

Thung (28) isolated a yellow mosaic believed to be similar to McKinney's (16) yellow mosaic of tobacco and Johnson's (13) tobacco virus

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No. 6. When approximately equal parts of juice containing the yellow-mosaic virus and juice containing the common tobacco-mosaic virus were mixed and the mixtures used to inoculate healthy tobacco plants, the symptoms of both diseases appeared in the inoculated plants. From certain spots in the leaves the infective principle of each disease was separately recoverable. If a light yellow spot was cut out of a leaf and used in the inoculation of healthy plants, the yellow mosaic was always recovered. If a bit of tissue from a darker green area was used, the ordinary green mosaic was isolated. This led Thung to conclude that only one virus could occupy any given cell. When a plant having yellow mosaic was inoculated with tobacco-mosaic virus, it failed to develop the symptoms of this disease. Likewise, when a plant having tobacco mosaic was inoculated with yellow-mosaic virus, the symptoms of the latter failed to appear. A single exception is mentioned. One plant having tobacco mosaic at the time it was inoculated with yellow-mosaic virus developed the symptoms of both diseases. Thung's work indicates that either, but only one, of the two viruses can multiply extensively in any given cell. His only means of testing for the presence or absence of either virus was to subinoculate to healthy tobacco plants and await the appearance of symptoms. He could easily prove by this method that the two viruses were not present in equal proportions in any selected group of cells. But he had no way of showing whether or not small amounts of either virus might be present in cells containing large amounts of the other.

Price (17) found that plants of the four species, *Nicotiana langsdorffii* Schrank, *N. sylvestris* Spegaz. and Comes, *N. quadrivalvis* Pursh., and *N. tabacum* L., regularly recover and are subsequently immune from the ring spot of tobacco. Although most recovered plants could not be distinguished from those that had not suffered an attack, they were invariably found to harbor the virus of ring spot. Juice from such plants proved to be highly infectious. Price showed that immunity from ring spot persists through at least three generations grown from cuttings but is not transmitted through seeds. The symptoms of tobacco mosaic in recovered plants were observed to be less severe than in plants free of the ring-spot virus.

Salaman (18) has recently reported the isolation of two strains of the X virus of potato. One strain, designated as the L type, causes a severe disease with intense mottling and dwarfing. The other, described as the G type, gives an almost imperceptible reaction. In both tobacco and *Datura stramonium*, the mild G type virus protects plants against infection by the severe L type virus. It does not, however, protect them against infection by the Y virus of potato or by the virus of ordinary tobacco mosaic. Here again is evidence that two closely related strains of a virus can not occupy the same plant tissue, although they are not antagonistic to unrelated viruses.

Botjes (4) has shown that when plants of certain potato varieties are infected with either secondary topnecrosis or attenuated secondary topnecrosis, they are, as a rule, protected against primary topnecrosis even though inoculated with the virus of this disease by means of grafting. The former produce mild symptoms and are apparently attenuated strains of the latter. Botjes' work brings evidence that potato plants infected by attenuated strains of topnecrosis are not invaded by unattenuated virus except in a small percentage of cases. Botjes states that the virus of topnecrosis is known in America as the healthy-potato virus. Since Kenneth Smith (27) believes his X virus to be one of the so-called healthy potato viruses first described by Johnson (11), it is possible that Salaman and Botjes worked with strains of the same disease.

East (10) has suggested that recovery of sugar-cane plants from mosaic may be due to acquired immunity, and has reported experiments believed to support this view.

Insofar as the writer is aware, the best evidence at present available in support of a belief that plants may acquire immunity from a virosis by virtue of having suffered an attack of that or of some closely related virosis, is found in the papers cited above. Thung, Salaman, and Botjes obtained evidence of acquired immunity in plants by making use of related strains of virus diseases in cross-immunization tests. Price obtained similar evidence by using a disease from which plants regularly recover.

Silberschmidt (24) made extensive studies on the problem of resistance to and immunity from tobacco mosaic. He was unable to obtain evidence of antibodies or to demonstrate either active or passive immunity in the plants studied.

Birkeland (3) reports experiments indicating that *Nicotiana glutinosa* L. does not develop an acquired immunity from infection by either the virus of tobacco mosaic or that of spot necrosis. Plants infected with tobacco mosaic and tested with spot necrosis, as well as plants infected with spot necrosis and tested with tobacco mosaic, gave similar results.

On Aucuba Mosaic of Tomato. Aucuba mosaic of tomato was first reported by Bewley (2) in 1924. He noted that it causes more intense yellowing of foliage than does ordinary tomato mosaic. He also observed that it differs from the latter in causing tomato fruits to mottle. The disease has been studied by Smith (25, 26), Sheffield (20, 21, 22, 23), and Caldwell (5, 6, 7, 8). Smith (25) has pointed out that, in its general characteristics and in the nature of the disease it produces, the virus of aucuba mosaic corresponds very closely to that of tobacco mosaic. He states that aucuba mosaic differs from the latter disease in the much greater intensity and brilliance of leaf symptoms but emphasizes the fact that it is similar to tobacco mosaic in filterability and in resistance to heat, alcohol,

dilution, and aging. He also notes a similarity in its ready transmissibility by inoculation with juice, as well as by insects. Later studies on filtration indicate, however, that the virus particle of aucuba mosaic is larger than that of tobacco mosaic (15).

The writer (14) has reported that aucuba mosaic differs from ordinary tobacco mosaic in its capacity to produce chlorotic primary lesions in the leaves of many varieties of tomato and chlorotic or necrotic primary lesions in the leaves of certain species and varieties of Nicotiana.

MATERIALS AND METHODS

The method of inoculation used in the experiments to be reported was the same in all cases, except where mention is made of a different method. Small glass spatulas similar to those described by Samuel (19) were used in applying inoculum. The flattened ends of the spatulas were covered by a layer of cheesecloth to prevent too much wounding of the leaf surfaces inoculated. The cloth was stretched tightly and smoothly over the ends of the spatulas and tied on with thread. All spatulas were covered with new cloth and sterilized in an autoclave before being used. Each inoculum was prepared by grinding small pieces of leaf tissue in a sterile mortar. Juice thus obtained was used to wet the cloth-covered ends of the spatulas. Except where otherwise stated, plants were inoculated by gently rubbing fresh juice from diseased plants of *Nicotiana tabacum* over the entire upper surface of a mature young leaf. After each inoculation excess inoculum was removed by washing the rubbed parts in a spray of water.

The plants were grown in soil in clay pots immersed in moist peat on benches in a greenhouse. The greenhouse was fumigated at frequent intervals to control insects. Before inoculation all plants were moved from the house in which they had been grown to an experimental greenhouse. Most of the experiments were made in the summer and autumn when the greenhouses were not heated, except for a few days in September and October. The temperature in the houses varied over a wide range, but is believed to have gone no lower than 65° F.

The plants used were in good growing condition. They were all of the species *Nicotiana sylvestris*, except where special mention is made of other species. The tobacco-mosaic virus was that designated by Johnson (13) as tobacco virus No. 1. It was obtained from James Johnson several years ago. The virus had been passed through tobacco plants many times before being used in the experiments here reported. The aucuba-mosaic virus was obtained from Cornell University through the kindness of H. H. Whetzel who got it from the Rothamsted Experimental Station, Harpenden, England. It also had been passed through tobacco plants many times before being used in the tests reported here.

The experiments to be described below have all been repeated one or more times. The results obtained in the repetitions agree closely with those reported.

THE SYMPTOMS OF TOBACCO AND AUCUBA MOSAICS ON NICOTIANA SYLVESTRIS

The mottling caused by tobacco and aucuba mosaics on most varieties of tobacco and tomato are much alike, except that aucuba mosaic produces leaf symptoms of greater brilliance and severity than those produced by tobacco mosaic. On mature leaves of *Nicotiana glutinosa, N. langsdorffii, N. rustica* L., *N. alata* Link and Otto, *N. sanderae* Sander, and the bean (*Phaseolus vulgaris* L.) varieties Refugee Green Pod, Robust, Scotia, Early Golden Cluster, Hodson Long Pod, and Ideal Market, the viruses of tobacco and aucuba mosaics produce necrotic primary lesions that are indistinguishable. On similar leaves of *N. sylvestris* and *N. tabacum*, varieties Adcock, Burley, and Connecticut Seed Leaf the virus of aucuba mosaic produces necrotic primary lesions, while that of tobacco mosaic causes faintly chlorotic primary lesions. Since systemic infection is associated with chlorotic primary lesions and does not ordinarily follow the production of necrotic primary lesions, the two diseases differ very sharply in their effects on these hosts.

The symptoms of tobacco mosaic on tobacco and a number of other host plants are recorded in a voluminous literature. Smith (25) has published an excellent description of aucuba mosaic on the tomato variety Kondine Red. The symptoms reported are similar to those shown by most other plants in which the disease becomes systemic. Any further general description is unnecessary. Since, however, the results of the experiments to be reported depend on the reaction of *Nicotiana sylvestris* to the viruses of tobacco and aucuba mosaics, a brief description of the symptoms of each disease as it appears on this host will be given.

Approximately four days after inoculation with tobacco-mosaic virus, primary lesions consisting of faint yellow spots appear on the leaf into which virus was rubbed. The spots vary in size from 1 to 2 mm. when first discernible. They enlarge gradually from day to day and, if numerous, may cause yellowing of the entire leaf. The intensity of yellowing in the primary lesions increases with the age of the lesions and the age of the leaf. The first symptom of systemic infection with tobacco mosaic is a clearing-of-veins in one or more leaves near the top of the plant. This is soon followed by mottling in young leaves. The old leaves are not mottled by the disease. The systemic symptoms are similar to those that have been described frequently for tobacco mosaic on tobacco.

If a young plant of *Nicotiana sylvestris* about 50 days' old is infected with the virus of aucuba mosaic by means of leaf inoculations, a result very

different from that following infection by tobacco mosaic is obtained. Necrotic primary lesions appear on the inoculated leaf in from one to two days, but all other leaves remain healthy in appearance. The necrotic lesions enlarge slowly but continuously until the leaf dies because of the enlarging lesions or of old age. If lesions are numerous, the leaf may collapse within 3 to 5 days after inoculation. The disease does not become systemic. All parts of the plant remain free of virus except the inoculated leaf. The differences in the symptoms produced by the two viruses are very conspicuous in *N. sylvestris*. Advantage may be taken of this fact to determine whether any sample of juice contains either or both of the viruses under study. The difference in the reaction of *N. sylvestris* and a few other plants to these two closely related viruses is unusual. A somewhat similar case has, however, been reported by Ainsworth (1).

ATTENUATED STRAINS OF AUCUBA MOSAIC

Aucuba mosaic has been maintained on tobacco and tomato for more than 2 years without change in symptoms. During this period the disease has been transmitted many times to *Nicotiana sylvestris*. When inoculated into the leaves of this plant, aucuba mosaic invariably has produced the necrotic primary lesions described above. It does not produce a general infection except in very young plants where it sometimes causes systemic necrosis. It never causes mottling. The conclusion that the disease does not vary and is due to a stable type of virus that may be maintained indefinitely is difficult to avoid.

It must be noted, however, that aucuba mosaic will become systemic if inoculated plants are held at a sufficiently high temperature for several days. Neither the minimum temperature nor the minimum time necessary to bring this about have yet been accurately determined. Young plants held at a temperature of about 35° C. for 3 days following infection with aucuba mosaic, invariably come down with a systemic form of the disease. When such plants are removed to greenhouses held at a temperature too low to initiate systemic infection, the systemic form of the disease persists. Three very distinct types or strains of aucuba mosaic have been obtained from plants incubated at high temperatures for various periods of time after inoculation.

The first strain closely resembles aucuba mosaic when transmitted to tobacco and tomato plants. But when inoculated into plants of *Nicotiana sylvestris*, it causes intense mottling with brilliant leaf symptoms such as are shown in figure 1, A. It does not produce necrotic primary lesions. It has been passed through *N. sylvestris* plants in serial transfers without apparent change. It has acquired the capacity to cause systemic infection, but has at the same time lost the ability to produce necrotic primary lesions.

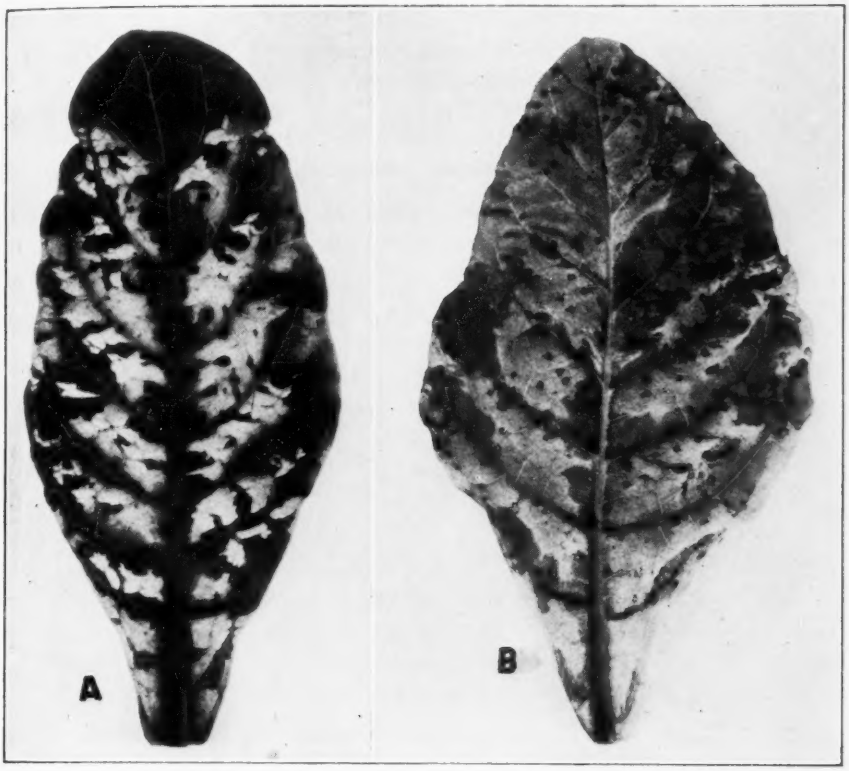

FIG. 1. A. Young leaf of *N. sylvestris* showing brilliant mottling due to an attenuated strain of aucuba mosaic. B. Leaf from a *N. sylvestris* plant having cucumber mosaic 5 days after it was inoculated with aucuba mosaic. Necrotic lesions have developed in both the light and dark green areas of the leaf. Cucumber mosaic does not give protection against aucuba mosaic.

The second strain differs from aucuba mosaic in that it does not produce on tobacco or tomato the brilliant leaf symptoms so characteristic of this disease. In fact, it is difficult to distinguish from tobacco mosaic on these plants. But when inoculated into *Nicotiana sylvestris*, it produces necrotic primary lesions typical of aucuba mosaic, and does not cause systemic infection.

The third strain differs very little from tobacco mosaic. On tomato and tobacco plants it causes a green mottling that is difficult to distinguish from tobacco mosaic. On *Nicotiana sylvestris* it produces no necrotic primary lesions, but causes mottling of the green mosaic type.

A number of other forms of aucuba mosaic have been obtained that may be characterized as intermediate strains. They will not be described here. All strains, including the 3 above described, are less severe than aucuba

mosaic on both tobacco and tomato plants. It is believed that the strains were derived from aucuba mosaic through attenuation by heat. They are, therefore, referred to as attenuated strains.

LEAVES MOTTLED BY ATTENUATED STRAINS OF AUCUBA MOSAIC IMMUNE FROM INFECTION BY UNATTENUATED VIRUS

Many tests have shown that mature mottled leaves of *Nicotiana sylvestris* plants, having either of the systemic attenuated forms of aucuba mosaic described above, are immune from unattenuated virus. When such leaves are rubbed with juice containing the virus of aucuba mosaic, no lesions whatever are produced, although a like inoculation of similar leaves of healthy plants invariably results in the production of numerous conspicuous necrotic lesions. The following experiment will serve to illustrate the manner in which the tests were made.

A mature mottled leaf on each of 10 plants infected with an attenuated strain of aucuba mosaic was rubbed with juice containing unattenuated virus. A similar leaf on each of 10 healthy plants was rubbed with another sample of the same inoculum. When the leaves were observed 4 days after their inoculation, it was found that numerous necrotic lesions had developed on all inoculated leaves of the previously healthy plants, but no lesions had developed on any of the mottled leaves. The experiment proves that leaf tissues mottled by attenuated virus become immune from infection by unattenuated virus.

MATURE LEAVES MOTTLED BY TOBACCO MOSAIC IMMUNE FROM AUCUBA MOSAIC

An experiment was undertaken for the purpose of determining whether the leaves of plants having tobacco mosaic might also acquire immunity from aucuba mosaic.

Each of 10 plants of *Nicotiana sylvestris* that had been infected with tobacco mosaic 57 days previous to the date on which they were selected for use in this experiment was inoculated by rubbing the virus of aucuba mosaic over the upper surface of each of 5 mature leaves. Each of 10 healthy plants of the same kind and age was inoculated in the same way. Ten similar plants left uninoculated served as checks. At the time of inoculation, the leaves of the plants having tobacco mosaic were typically mottled, slightly malformed, and somewhat stunted. In other respects they were similar to the leaves of the healthy plants. Both sets of leaves were approximately the same age.

Four days after their inoculation with the virus of aucuba mosaic the 50 inoculated leaves on the 10 previously healthy plants showed numerous necrotic lesions, such as are typical of the primary lesions produced by

this disease. The 50 mottled leaves on the 10 plants having tobacco mosaic were not visibly affected. No lesions of any kind developed on these leaves during the period of 6 weeks that they were held under observation. The uninoculated check plants remained healthy. Figure 2 shows a leaf from each set of the inoculated plants as it appeared 5 days after inoculation. The test proves that mature leaves of *Nicotiana sylvestris* plants having tobacco mosaic are immune from aucuba mosaic under the conditions of the experiment.

FIG. 2. Two leaves of *N. sylvestris* as they appeared 5 days after both were inoculated with aucuba mosaic. The leaf on the left was healthy, while that on the right was mottled by tobacco mosaic at the time of inoculation with aucuba mosaic.

In order to determine whether the virus of aucuba mosaic might have infected and multiplied in the mottled leaves without producing lesions, subinoculations were made from each of 2 of the inoculated leaves on each

of the 20 plants 7 days after their inoculation with aucuba mosaic. The subinoculations were made from discs of leaf tissue about 1 cm. in diameter, cut out of each of the leaves by means of a sterile cork border. Each disc was mashed in a few drops of water on a glass slide. The inoculum thus obtained was used to inoculate 2 leaves on each of 40 healthy young plants of *Nicotiana sylvestris*. Subinoculations from the 20 mottled leaves produced only 4 necrotic lesions. One lesion developed from inoculations with juice obtained from each of 4 discs. These lesions may have resulted from virus left on the surface of the discs when the leaves were inoculated 7 days earlier. The subinoculations from the 20 leaves bearing lesions produced necrotic lesions in every instance. A total of 127 lesions resulted from these inoculations. The subinoculations prove that the virus of aucuba mosaic multiplies little or none in the mottled leaves.

YOUNG LEAVES OF PLANTS HAVING TOBACCO MOSAIC NOT IMMUNE FROM AUCUBA MOSAIC

An experiment was made for the purpose of determining whether very young leaves of plants having tobacco mosaic are immune from aucuba mosaic.

Ten healthy young plants were inoculated with tobacco-mosaic virus. Ten similar plants left uninoculated served as checks. All of the 10 inoculated plants came down with tobacco mosaic. The 10 check plants remained healthy. Two weeks after the first appearance of symptoms in the tips of the plants, a young mottled leaf, about 1½ in. long, on each of the 10 plants was inoculated by rubbing the upper surface with juice containing the virus of aucuba mosaic. Similar leaves on the 10 check plants were likewise inoculated. One week after these inoculations, several necrotic lesions were observed on each of the mottled leaves. Most, but not all, of the lesions were on veins. Typical aucuba-mosaic lesions appeared on the inoculated leaves of all the check plants. They were, however, more numerous on these leaves than on the mottled leaves. The lesions on both the mottled and the check leaves enlarged slowly. Three weeks after their appearance, subinoculations were made from 10 of the necrotic lesions on mottled leaves to leaves of healthy young *Nicotiana sylvestris* plants. All of the subinoculations resulted in the production of necrotic spots typical of the primary lesions of aucuba mosaic. A total of 78 lesions resulted. The test brings evidence that the virus of aucuba mosaic multiplies in lesions on mottled leaves and proves that very young leaves of plants having tobacco mosaic are not immune from aucuba mosaic. The young leaves differ distinctly from mature leaves in this respect.

IMMUNIZATION BY DIRECT LOCAL INOCULATION OF MATURE LEAVES

On some of the plants used in the experiments described above, it was observed that leaves inoculated with tobacco-mosaic virus previous to inoculation with the virus of aucuba mosaic, bore fewer necrotic lesions than the other mature green leaves on the same plants. This suggested the possibility of immunizing mature leaves by direct inoculation. Experiments were, therefore, undertaken to determine whether the direct inoculation of full-grown leaves with the virus of tobacco mosaic would render them immune from aucuba mosaic.

A mature leaf on each of 10 healthy plants was inoculated by rubbing

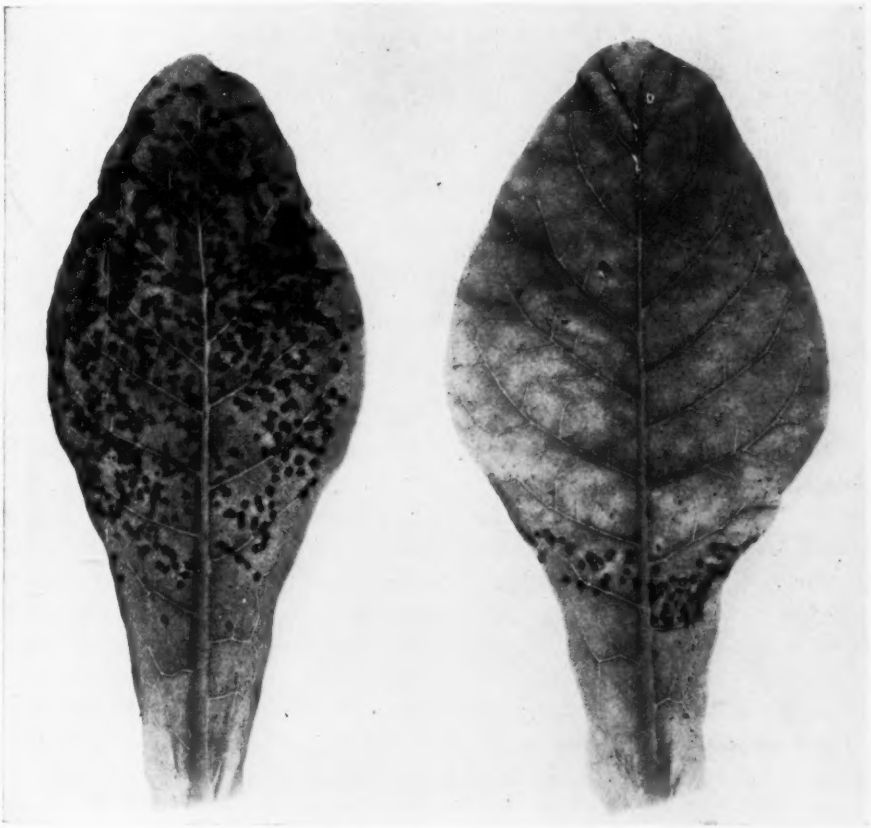

FIG. 3. Two leaves of *N. sylvestris*. The leaf on the right was rubbed with juice containing the virus of tobacco mosaic 5 days before both were inoculated equally with aucuba mosaic. The leaf on the right acquired immunity from aucuba mosaic as is shown by the absence of primary lesions in most of the tissues.

juice containing the virus of tobacco mosaic over the entire upper surface. Another mature leaf on each plant was rubbed with juice from a healthy plant. This was done in order to cause approximately the same degree of wounding on both sets of leaves. Five days later, all of the leaves were reinoculated by rubbing juice containing the virus of aucuba mosaic over their entire upper surfaces. When the leaves were examined 4 days after this inoculation, it was found that many necrotic spots typical of the primary lesions of aucuba mosaic had developed on the leaves rubbed with virus-free juice before they were inoculated, whereas very few necrotic spots had developed on the leaves inoculated with the virus of tobacco mosaic previous to inoculation with that of aucuba mosaic. It was observed that most of the necrotic lesions occurring on the leaves inoculated with both viruses were confined to areas that might have been missed in the first inoculation. Most of them were on the bases of the leaves where the second inoculation may have extended somewhat lower than the first. A set of leaves from one of the plants is shown in figure 3. The leaf on the left was rubbed with juice from a healthy plant, that on the right with juice from a plant having tobacco mosaic, 5 days before both leaves were inoculated equally with aucuba mosaic. It will be seen that necrotic lesions are well distributed over the leaf rubbed with healthy juice, but are confined chiefly to the base of the leaf rubbed with juice containing tobacco-mosaic virus. The experiment proves that mature leaves inoculated with tobacco-mosaic virus acquire immunity from aucuba mosaic.

IMMUNITY CLOSELY ASSOCIATED WITH INVASION BY THE VIRUS OF TOBACCO MOSAIC

In the description of tobacco mosaic on *Nicotiana sylvestris*, it was pointed out that mottling occurs only in those leaves that are very young at the time of infection. Old leaves are not mottled by the spread and multiplication of the virus. It also was pointed out that one or more leaves that are young at the time of infection may show the symptom described as clearing-of-veins. This symptom frequently occurs in the base of a leaf only. In that case the tip retains its normal green color and is apparently unaffected.

It was of interest to determine whether immunity from the virus of aucuba mosaic would be confined to mottled leaves or would extend to unaffected leaves or leaves with clearing-of-veins.

Each of 10 healthy young plants was inoculated by rubbing the virus of tobacco mosaic over the upper surface of a single leaf. Ten similar plants were left uninoculated and served as checks. Two weeks after inoculation, when one or more of the mottled leaves on each inoculated plant had reached maturity, all full-grown leaves were tagged with labels indi-

eating whether they were mottled, apparently healthy, or whether they showed clearing-of-veins. All leaves that were only partly affected by the clearing-of-veins symptom were marked with dotted lines to indicate exactly what portions were unaffected. The lines were drawn with India ink applied with a camel-hair brush. They were drawn across the upper surfaces of the leaves in such a way as to mark off the portions that were unaffected from those showing chlorosis. Since there is usually a zone of slightly discolored tissue between the areas with marked clearing-of-veins and those of a normal green color, it was not always easy to determine where the line separating affected from nonaffected parts should be drawn. All mature leaves of the 10 plants were heavily inoculated with the virus of aucuba mosaic. Similar leaves on 5 of the check plants were likewise inoculated. The remaining 5 check plants were again left uninoculated.

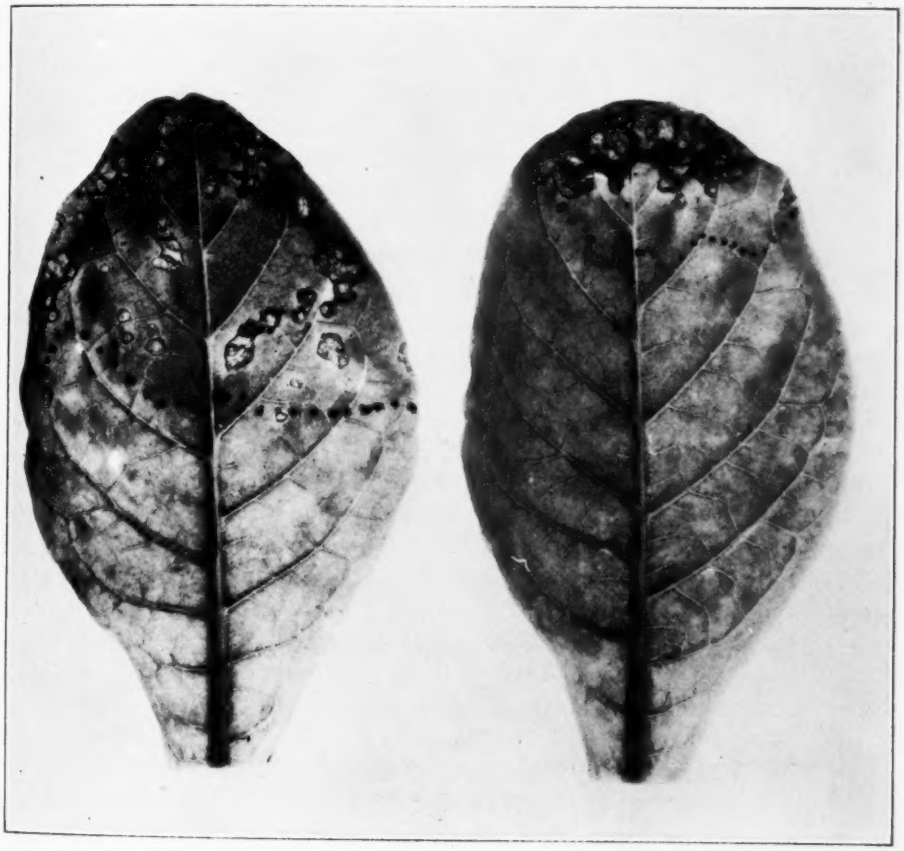

FIG. 4. Two leaves of *N. sylvestris* partly invaded by the virus of tobacco mosaic as judged by the clearing-of-veins symptom. The invaded portions are immune from aucuba mosaic as is shown by the failure of lesions to develop in these portions.

When the plants were observed 5 days after these inoculations, it was found that numerous necrotic spots, such as are typical of the primary lesions of aucuba mosaic on leaves of *Nicotiana sylvestris*, had developed on all the inoculated leaves of the 5 check plants and on all the normal green leaves of the 10 plants having tobacco mosaic. All the mottled leaves, as well as all leaves showing the clearing-of-veins symptom throughout, were free of lesions. The 5 uninoculated check plants were likewise free of lesions. The leaves bearing India ink lines that marked off areas with clearing-of-veins from areas unaffected by the tobacco mosaic showed practically no lesions in the chlorotic areas below the lines but numerous lesions in the normal green areas above the lines. A few lesions occurred on, or slightly below, the lines in some of the leaves. Since the entire upper surfaces of the leaves were inoculated equally with the virus of aucuba mosaic, it is evident that only those portions visibly affected by tobacco mosaic become immune from aucuba mosaic during the first 2 weeks following inoculation. In several of the leaves, however, narrow zones of lesion-free areas just above the ink lines were observed. Two leaves inoculated with the virus of aucuba mosaic after they had been partly invaded by tobacco mosaic are shown in figure 4. The leaf on the left bears one necrotic lesion just below the inked line. It is probable that the line was incorrectly drawn at the point where the lesion occurs.

Several experiments were made for the purpose of determining whether old leaves that remain entirely free of symptoms acquire immunity from aucuba mosaic. At various intervals after the first appearance of symptoms in young plants, leaves too old to show clearing-of-veins were inoculated by rubbing their upper surfaces with the virus of aucuba mosaic. It any symptoms of tobacco mosaic, become immune from aucuba mosaic. was found that old leaves that remain deep green in color and never show Immunity extends far beyond the parts visibly affected by tobacco mosaic. It is, however, acquired very slowly by the old leaves. Parts of some of them remain susceptible over a period of one or two months following inoculation with tobacco-mosaic virus. Numerous tests have shown that the virus of tobacco mosaic moves into old leaves very slowly. Such leaves acquire immunity from aucuba mosaic soon after being invaded by the virus of tobacco mosaic. The experiments prove that immunity from the former is closely associated with invasion by the latter.

TIME REQUIRED FOR THE DEVELOPMENT OF IMMUNITY FOLLOWING SYSTEMIC INFECTION

An experiment was made for the purpose of determining the time required for the development of immunity in young leaves invaded in the course of systemic infection.

Each of 21 healthy young plants was inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of a single leaf. The plants were then separated into 7 groups of 3 plants each. After one day, and on each successive day over a period of a week, one group of plants was reinoculated by rubbing juice containing the virus of aucuba mosaic over the upper surface of all leaves except those previously inoculated with tobacco-mosaic virus and the young leaves less than 2 in. long. The plants were examined 5 days after the last group was inoculated.

Numerous necrotic lesions appeared on all parts of the leaves of the plants in the first and second groups. In the third group half of a leaf on one plant and about one-third of each of 2 leaves on the other 2 plants were free of lesions. Parts of one or more leaves on each of the plants of the fourth group were likewise free of lesions. In the fifth group large parts of leaves on each of the 3 plants were entirely free of lesions, and one leaf on one plant was lesion-free. One or more leaves on each plant in the sixth and seventh groups were also entirely free of lesions.

The experiment shows that no protection whatever is afforded by systemic infection during the first 2 days following inoculation. Parts of several leaves were protected on the third and fourth days. Not until the fifth day following inoculation was any leaf wholly protected.

The movement of tobacco mosaic virus in *Nicotiana sylvestris* plants may be accurately followed by means of immunity tests. Small quantities of virus do not confer immunity, but the tissues become immune soon after invasion.

TIME REQUIRED FOR THE DEVELOPMENT OF IMMUNITY IN LEAVES FOLLOWING DIRECT INOCULATION

It was found that very little protection is afforded by the inoculation of healthy leaves of *Nicotiana sylvestris* immediately before their exposure to aucuba mosaic. An experiment was undertaken for the purpose of determining the time necessary for the development of immunity.

Twenty-four healthy young plants were divided into 8 groups of 3 plants each. One mature leaf on each plant was inoculated by rubbing fresh juice containing the virus of tobacco mosaic over the entire upper surface. A similar leaf on each plant was rubbed with water, it having been shown to be immaterial whether the uninoculated leaves are rubbed with water or with juice from healthy plants. Immediately after this inoculation and on each succeeding day for the following 7 days one group of plants was inoculated by rubbing juice containing the virus of aucuba mosaic over the upper surface of each leaf previously rubbed with juice containing the virus of tobacco mosaic or with water. Four days after the last group was inoculated, all plants were carefully observed. A count or an estimate of the

number of lesions that developed on each leaf was made. The results obtained are recorded in table 1. The table shows that little protection is afforded by rubbing the leaves with virus of tobacco mosaic immediately preceding their inoculation with the virus of aucuba mosaic. After an interval of one day, however, more than twice as many lesions developed in the unprotected as in the protected leaves. After a period of 2 days or

TABLE 1.—*Time required for development of immunity in the leaves of Nicotiana sylvestris following direct inoculation.*

Interval in days between inoculation with viruses of tobacco mosaic and aucuba mosaic	Plant	Number of lesions on leaves rubbed with tobacco-mosaic virus before inoculation with virus of aucuba mosaic	Number of lesions on leaves rubbed with water before inoculation with aucuba-mosaic virus
0	1	239	406
	2	500[a]	560[a]
	3	350[a]	450[a]
1	4	42	275[a]
	5	125[a]	325[a]
	6	150[a]	400[a]
2	7	0	325[a]
	8	4	400[a]
	9	9	350[a]
3	10	6	325[a]
	11	5	356
	12	5	300[a]
4	13	1	526
	14	3	450[a]
	15	0	350[a]
5	16	0	300
	17	0	350[a]
	18	0	425[a]
6	19	0	325[a]
	20	6	350[a]
	21		
7	22	0	250[a]
	23	0	375[a]
	24	10	225[a]

[a] Number estimated.

longer few or no lesions developed in the protected leaves. The distribution of the few that did appear was such as to suggest that they developed in areas that escaped inoculation with tobacco-mosaic virus. They were located on the bases or edges of the leaves. Other parts of the leaves were entirely free of lesions. The experiment proves that two days are sufficient

for the development of immunity in leaves directly inoculated with the virus of tobacco mosaic.

IMMUNITY CLOSELY CONFINED TO AREAS INOCULATED

The peculiar distribution of the lesions of aucuba mosaic on leaves rubbed with the virus of tobacco mosaic suggested that an experiment should be made to determine whether immunity is closely confined to inoculated leaf areas or may extend somewhat beyond them. The following experiment was undertaken.

Five mature leaves on each of 5 healthy *Nicotiana sylvestris* plants were selected for inoculation. Juice containing tobacco-mosaic virus was rubbed over the upper surface of one side of each leaf. The upper surface of the other side was rubbed with juice from a healthy plant. An effort was made to rub the 2 sides equally, so that trichomes and upper epidermal cells on both sides would be wounded to approximately the same extent. Three days after this inoculation the leaves were given a second inoculation by rubbing juice containing the virus of aucuba mosaic over their entire upper surfaces. Four days after this second inoculation it was found that an average of about 200 necrotic spots, typical of the primary lesions of aucuba mosaic, had developed on the sides of the leaves previously rubbed with virus-free juice, whereas an average of only 12 necrotic spots had developed on the sides rubbed with juice containing tobacco-mosaic virus. On 5 of the leaves no lesions whatever occurred on the sides inoculated with tobacco mosaic. The lesions were rather evenly distributed over the sides rubbed with virus-free juice, but very unevenly distributed over the sides rubbed with tobacco-mosaic virus. Most of the lesions were confined to the edges and bases of these sides. Such areas may have escaped inoculation with the tobacco-mosaic virus. There was no indication that inoculation of half of a leaf with tobacco-mosaic virus affects in the slightest degree the susceptibility of the other half to the virus of aucuba mosaic over the period tested. The experiment proves that leaf areas inoculated with the virus of tobacco mosaic acquire immunity from the virus of aucuba mosaic, but that adjacent areas not so inoculated remain susceptible. Immunity does not extend much beyond the areas inoculated.

HEAVY INOCULATION NECESSARY FOR THE COMPLETE IMMUNIZATION OF MATURE LEAVES

In the experiment described above, only 5 of the leaves remained entirely free of necrotic lesions on the sides rubbed with tobacco-mosaic virus. This suggested that a very thorough inoculation with tobacco-mosaic virus may be necessary to obtain complete protection against aucuba mosaic. An attempt was made to inoculate certain parts of leaves more completely and

more heavily with the tobacco-mosaic virus than was done in the foregoing experiments.

Forty-six mature leaves on 8 healthy young plants of *Nicotiana sylvestris* were inoculated with the virus of tobacco mosaic in the following manner. The upper surface of one side of each of 6 leaves on each of 2 plants was very thoroughly and evenly rubbed with fresh juice containing the virus of tobacco mosaic. Care was taken to avoid missing any part of the leaf surface on the side being inoculated. The upper surface of the tip portion of each of 4 leaves on one plant, 6 leaves on another, and 7 leaves on a third, was thoroughly rubbed with another sample of the same inoculum. The upper surface of the basal portion of 5 leaves on each of 2 plants, and of 7 leaves on another plant, was similarly inoculated. The upper surfaces of the uninoculated portions of each of the leaves were just as thoroughly rubbed with water.

Five days after inoculation with the virus of tobacco mosaic, all leaves were given a second inoculation by rubbing the virus of aucuba mosaic over their entire upper surfaces. All portions of the leaves were inoculated equally with virus from the same sample of juice.

Three days after the second inoculation all leaves were carefully observed. The 12 inoculated with tobacco-mosaic virus on one side only were entirely free of nectrotic lesions on the inoculated sides. From 125 to 500 necrotic spots typical of the primary lesions of aucuba mosaic were present on the opposite sides. A careful estimate showed that about 3250 lesions had developed on the sides of the leaves not inoculated with tobacco-mosaic virus. The sides heavily inoculated were completely protected against aucuba mosaic.

Of the 17 leaves inoculated with tobacco-mosaic virus in their tips, 12 showed no necrotic lesions in the areas so inoculated. Two showed 1 lesion each, and one showed 2, one 4, and one 5 lesions in their tip areas. Necrotic lesions were well distributed over the basal portions of all of the 17 leaves. A count showed that there were about 2730 lesions on the basal portions of the 17 leaves.

Of the 17 leaves inoculated with tobacco-mosaic virus over their basal portions, 12 were entirely free of lesions in these halves. One showed 1 lesion, one 2 lesions, and three 5 lesions each, in the inoculated areas. Necrotic lesions were well distributed over the tip portions of all leaves. It was estimated that more than 5000 lesions were present on the tip halves of the 17 leaves.

A total of 31 necrotic lesions of aucuba mosaic developed in the protected areas of 10 leaves. No lesions whatever developed in the protected areas of the other 36 leaves. Approximately 11,000 lesions appeared in the unprotected halves of the 46 leaves. The experiment shows how fully

FIG. 5. Three leaves of *N. sylvestris* partly immunized from aucuba mosaic by direct inoculation with tobacco mosaic. The areas free of lesions were rubbed with juice containing the virus of tobacco mosaic 5 days before all parts of the leaves were inoculated equally with aucuba mosaic.

leaves, or portions of leaves, may be protected against aucuba mosaic by heavy inoculation with the virus of tobacco mosaic, and how closely protection is confined to the areas inoculated. A leaf taken from each set of plants used in this experiment is shown in figure 5. The left half of the leaf on the left, the lower half of the middle leaf, and the upper half of the leaf on the right were inoculated with the virus of tobacco mosaic 5 days before all parts of the upper surfaces of all leaves were inoculated equally with the virus of aucuba mosaic.

LOCAL PROTECTION NOT CONFINED TO CELLS ACTUALLY INOCULATED WITH TOBACCO-MOSAIC VIRUS

It is evident from the foregoing experiments that in mature leaves protection is rather closely confined to the areas inoculated. The following experiment was undertaken for the purpose of determining whether inoculation of the lower surfaces of mature leaf areas with tobacco-mosaic virus would protect such areas against subsequent infection with the virus of aucuba mosaic when inoculated into the upper surfaces.

Two mature leaves on each of 10 healthy young plants were inoculated by rubbing juice containing the virus of tobacco mosaic over the lower surface of one side of each leaf. The lower surface of the other side was in each case rubbed with water. Two similar leaves on each of the 10 plants

were inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of one side of each leaf. The upper surface of the other side of each leaf was similarly rubbed with water.

Four days after these inoculations, all of the leaves were inoculated a second time by rubbing fresh juice containing the virus of aucuba mosaic over their entire upper surfaces.

Five days after the second inoculation, the leaves were carefully observed. There was very little difference to be seen between the degree of protection obtained by inoculation of the lower surfaces, and that obtained by inoculation of the upper surfaces. The protection afforded by both methods was almost, but not quite, complete. A few lesions developed on the edges of the protected halves of most of the leaves. The experiment proves that protection extends somewhat beyond the cells actually inoculated. A microscopic examination of sections through the leaves showed them to have a minimum thickness of 7 cell layers. Five layers of cells intervened between the cells of the lower epidermis that were inoculated and those of the upper epidermis that were protected.

LITTLE OR NO MULTIPLICATION OF AUCUBA-MOSAIC VIRUS IN PROTECTED TISSUES

In the experiments described up to this point, no attempt was made to determine whether the protection due to direct inoculation of leaves with tobacco-mosaic virus consists in preventing infection by the virus of aucuba mosaic, or only in inhibiting the symptoms of this disease. An experiment was, therefore, made to determine whether the virus of aucuba mosaic multiplies in protected tissues.

Two mature leaves on each of 6 healthy young plants were inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of one side of each leaf. The other sides of the leaves were rubbed with water. Five days after this inoculation, all of the leaves were given a second inoculation by rubbing their entire upper surfaces with juice containing the virus of aucuba mosaic. When the leaves were observed 3 days after the second inoculation, it was found that numerous necrotic spots typical of the primary lesions of aucuba mosaic had developed on all of the leaves on the unprotected sides, whereas no lesions had developed on the protected sides. Two discs of tissue about 1 cm. in diameter were cut from each side of each of the 12 leaves by means of a sterile cork borer. Each disc was crushed in a few drops of water on a glass slide. The juice obtained from each of the 24 discs of unprotected tissue was used to inoculate one side of each of 24 mature leaves on 6 healthy *Nicotiana sylvestris* plants. The opposite sides of the leaves were inoculated with juice obtained from each of the 24 discs of protected tissue. The leaves were

observed 4 days after inoculation. A total of 414 lesions were counted on the sides inoculated with juice from unprotected tissue, but only 6 were found on the sides inoculated with juice from protected tissue. The experiment shows that little or no multiplication occurs in the protected tissues. The 6 lesions obtained by inoculating with juice from discs of protected tissue may have resulted from virus left on these discs when they were inoculated with aucuba mosaic.

THE IMMUNE REACTION IN PLANTS OF DIFFERENT AGES

An experiment was made to determine whether the leaves of old plants are as easily protected against aucuba mosaic as those of young ones. Nine healthy *Nicotiana sylvestris* plants 57 days old, nine 109 days old, and nine 165 days old were divided into 3 groups of 9 plants each. Three plants of each of the 3 different ages were placed in each group. The plants of the first group were inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of one side of each leaf large enough to inoculate; those of the second group by rubbing a like sample of juice over the upper surface of the tip portion of each leaf; and those of the third group by rubbing a similar inoculum over the upper surface of the basal portion of each leaf. The uninoculated parts of all leaves were rubbed with water. Three days after their inoculation with tobacco-mosaic virus

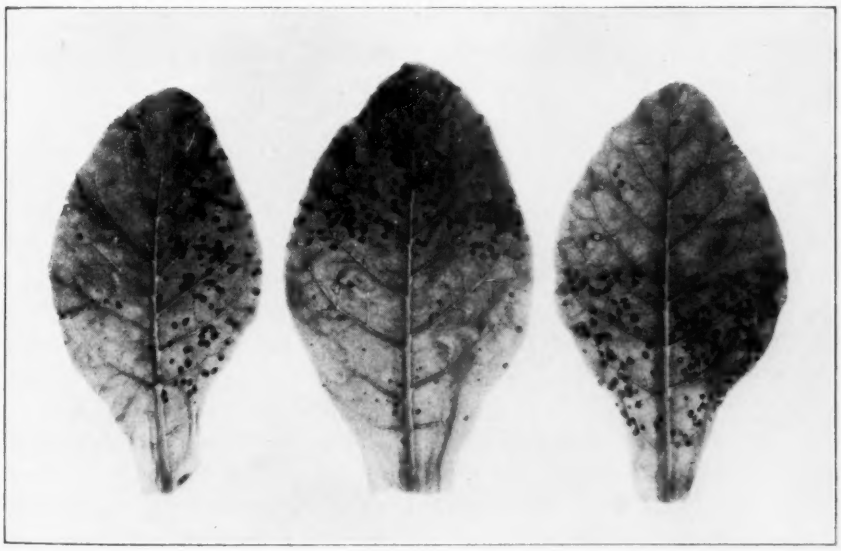

FIG. 6. Three leaves of *N. sylvestris* from plants 5½ months old. The areas bearing few lesions were rubbed with juice containing the virus of tobacco mosaic 3 days before all parts of the leaves were inoculated equally with aucuba mosaic.

all leaves were inoculated a second time by rubbing juice containing the virus of aucuba mosaic over their entire upper surfaces.

When the plants were observed 5 days after the second inoculation it was found that numerous necrotic lesions had developed on all of the unprotected portions of the leaves. A small number of lesions had also developed in the protected areas of many leaves. The leaves of the youngest plants were more completely protected than those of the other 2 sets. The best protection was obtained in the oldest leaves of the youngest plants. The poorest protection was obtained in leaves of the oldest plants, but, even in these, it was quite marked, as may be seen from figure 6. The picture shows a leaf from one of the oldest plants in each of the 3 groups. The experiment proves that inoculation with tobacco-mosaic virus affords local protection against aucuba mosaic in old as well as in young plants.

RECIPROCAL CROSS-IMMUNITY TESTS

A number of reciprocal cross-immunity tests were undertaken. The object of the tests was to determine whether inoculation of leaves with the virus of aucuba mosaic would render them immune from tobacco mosaic.

In a typical experiment, 3 leaves on each of 10 healthy young plants were inoculated with aucuba mosaic by means of needles. The needle-puncture method was used in preference to the rubbing method, because the number of points of infection is more easily controlled when the latter method is used. Each leaf received 100 punctures with needles dipped in juice containing the virus. On the fourth day following this inoculation, when necrotic lesions resulting from inoculations by the needle punctures had reached a diameter of $\frac{1}{4}$ cm., the leaves were reinoculated by rubbing one side of the upper surface of each with juice containing the virus of tobacco mosaic. The plants were held under observation for 3 weeks following this inoculation. The sides of the leaves inoculated with tobacco mosaic yellowed somewhat as a result of the development of numerous primary lesions at the points of infection with tobacco mosaic. The plants all came down with systemic infection. There was at no time any visible evidence that tissues adjacent to aucuba mosaic lesions had developed immunity from tobacco mosaic. The lesions produced by the latter were, however, too indistinct to be differentiated with certainty from yellowing due to other causes. Yellowing associated with the killing action of aucuba mosaic in tissues surrounding the lesions interferes with observations on the tobacco-mosaic lesions. The technique is inadequate for the purpose of demonstrating immunity in tissues surrounding these lesions. It is possible that suitable staining methods might show immunity. All that this and other similar experiments prove is that small numbers of aucuba mosaic lesions do not render leaves immune from tobacco mosaic.

IMMUNIZATION BY INOCULATION WITH ATTENUATED STRAINS OF TOBACCO MOSAIC

Following the method described by Johnson (12) for the attenuation of tobacco mosaic by heat, 3 attenuated strains of this virus were secured. They were isolated from 3 different tobacco plants incubated at about 35° C. for 15 days after being inoculated with tobacco mosaic. The 3 strains showed different degrees of attenuation as judged by the symptoms produced on tobacco and other plants. All caused milder symptoms than the virus from which they were obtained. The strain selected for use in the experiments to be reported produced a mild type of mottling and did not cause much stunting in either tobacco or *Nicotiana sylvestris* plants. Experiments were made to determine whether this attenuated strain would give protection against aucuba mosaic.

Ten healthy young plants were inoculated with tobacco mosaic. Ten similar plants were inoculated with the attenuated virus, and 10 were left uninoculated. After a period of 6 weeks, the plants inoculated with attenuated virus showed a mild type of mottling and were somewhat stunted in growth. They were, however, much larger than the plants inoculated with tobacco mosaic. Two of the youngest mature leaves of each of the 30 plants were then inoculated by rubbing fresh juice containing the virus of aucuba mosaic over their entire upper surfaces. Five days after this inoculation it was found that numerous necrotic spots typical of the primary lesions of aucuba mosaic had developed on each of the inoculated leaves of the 10 check plants. No lesions had developed on any of the leaves of the 10 plants inoculated with tobacco mosaic nor on the leaves of the 10 plants inoculated with the attenuated strain of this virus. Figure 7 shows a leaf from a healthy plant and one from a plant infected with attenuated virus, 5 days after both were inoculated equally with aucuba mosaic. The experiment proves that *Nicotiana sylvestris* leaves mottled by this attenuated virus are as completely protected against aucuba mosaic as similar leaves mottled by ordinary tobacco mosaic.

In another experiment, one side of one leaf on each of 10 healthy young *Nicotiana sylvestris* plants was heavily inoculated by rubbing juice containing the virus of tobacco mosaic over its upper surface. A similar leaf on each plant was inoculated by rubbing juice containing the attenuated virus over the upper surface of one side. The upper surfaces of the uninoculated sides of all of the leaves were rubbed with water. Five days after this inoculation all leaves were inoculated a second time by rubbing fresh juice containing the virus of aucuba mosaic over their entire upper surfaces. When the plants were observed on the sixth day following the second inoculation, it was found that numerous necrotic lesions of aucuba mosaic had

FIG. 7. Two leaves from *N. sylvestris* plants 5 days after both were inoculated equally with aucuba mosaic. The leaf on the left is from a plant infected with an attenuated strain of tobacco mosaic; that on the right from a healthy plant.

developed on the sides of the leaves rubbed with water, whereas very few lesions had developed on the sides inoculated with either tobacco-mosaic virus or with the attenuated strain of this virus. The sides of two of the leaves inoculated with tobacco-mosaic virus showed 5 lesions each. The corresponding sides of the other 8 leaves were free of lesions. The sides of 4 leaves inoculated with attenuated virus showed 1, 2, 4, and 5 lesions respectively. The corresponding sides of the other 6 leaves were free of lesions. A total of only 10 lesions appeared on the sides of the leaves inoculated with tobacco-mosaic virus. The opposite unprotected sides were estimated to bear about 3000 lesions. A total of 12 lesions appeared on the sides of the 10 leaves inoculated with attenuated virus. The opposite unprotected sides of the same leaves were estimated to bear about 2800 lesions.

The experiment brings evidence that even in mature leaves this attenuated strain of virus gives protection against aucuba mosaic equal to that afforded by ordinary tobacco mosaic. Similar tests of the other 2 attenuated strains gave evidence that they also afford the same protection.

PROTECTION OF PLANTS AGAINST TOBACCO MOSAIC BY ATTENUATED VIRUS

An experiment was made for the purpose of determining whether the immunity conferred by attenuated strains of tobacco mosaic would protect plants against tobacco as well as aucuba mosaic.

Twenty healthy young plants were inoculated with an attenuated strain of the virus of tobacco mosaic. Twenty similar plants left uninoculated served as checks. When, after a period of one week, the inoculated plants were showing early symptoms of infection, 10 of them were reinoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of the first leaf above that into which attenuated virus was rubbed. Ten of the healthy plants were similarly inoculated. All of the plants were held under observation for one month after the tobacco mosaic inoculations were made. The 10 uninoculated check plants remained healthy. The 10 plants inoculated with attenuated virus, only, came down with the symptoms typical of the disease produced by this virus. The leaves were somewhat mottled but not distorted. The plants were slightly stunted. The 10 plants inoculated with tobacco mosaic only, came down with tobacco mosaic. On the fifth day following inoculation, one or more leaves on each plant showed the clearing-of-veins symptom. This was followed by mottling and distortion of leaves. The plants were badly stunted when the experiment was ended. The 10 plants inoculated with tobacco-mosaic virus one week after being inoculated with attenuated virus, came down with symptoms characteristic of the disease caused by the latter. At no time did any of them show the symptoms of tobacco mosaic. The leaves were not severely mottled and the plants only slightly stunted. These plants were, in fact, indistinguishable from those inoculated with attenuated virus only. The experiment proves that attenuated virus may be used for the protection of plants against tobacco mosaic.

SPECIFICITY OF THE IMMUNE REACTION

In order to secure evidence as to the specificity of protection by inoculation of plants or individual leaves with tobacco-mosaic virus or its attenuated strains, experiments were made for the purpose of determining whether cucumber mosaic or the ring-spot of tobacco might give protection against aucuba mosaic.

Each of 10 plants that had shown the symptoms of cucumber mosaic for

about 5 weeks was inoculated by rubbing juice containing the virus of aucuba mosaic over the upper surface of 2 full-grown mottled leaves. Ten other plants that had had tobacco mosaic for a like period of time were similarly inoculated. Two leaves on each of 10 healthy plants were likewise inoculated and served as checks. When the leaves were examined 5 days after these inoculations it was found that necrotic lesions had developed on all of the check leaves and on all of the leaves mottled by cucumber mosaic. No lesions whatever had developed on the leaves mottled by tobacco mosaic. A typical leaf from one of the plants having cucumber mosaic is shown in figure 1, B. The lesions of aucuba mosaic occur in the light green, as well as in the dark green, areas of the leaf. The experiment proves that the systemic infection of leaves by cucumber mosaic does not protect them against aucuba mosaic.

As is well known from the work of Price (17), *Nicotiana sylvestris* plants regularly recover and are subsequently immune from ring-spot of tobacco. An experiment was made for the purpose of determining the reaction of recovered plants to aucuba mosaic.

Each of 10 plants that had suffered an attack of ring spot and had recovered to such an extent that their tips, including several mature leaves, appeared to be healthy were inoculated by rubbing fresh juice containing the virus of aucuba mosaic over the upper surface of 2 of the youngest mature leaves. Two mottled leaves on each of 10 plants having tobacco mosaic were similarly inoculated. Two leaves on each of 10 healthy plants inoculated in the same manner served as checks.

On the fifth day following these inoculations, it was found that numerous necrotic lesions had developed on all of the check leaves. An approximately equal number had developed on the inoculated leaves of the plants that had recovered from ring spot. No lesions whatever appeared on the leaves mottled by tobacco mosaic. The experiment proves that leaves of plants that have recovered from ring spot and are immune from this disease are not thereby protected against aucuba mosaic, although, under the conditions of the experiment, similar plants having tobacco mosaic are fully protected against this disease.

An experiment was made for the purpose of determining whether mature leaves of *Nicotiana sylvestris* can be protected against aucuba mosaic by direct inoculation with either the virus of cucumber mosaic or that of ring spot.

A mature leaf on each of 10 healthy young plants was inoculated by rubbing juice containing the virus of cucumber mosaic over its entire upper surface. A second, third, and fourth leaf on each plant was rubbed with juice containing the virus of ring spot, juice containing the virus of tobacco mosaic, and water, respectively. Five days after these inoculations all of the leaves were rubbed with juice containing the virus of aucuba mosaic.

On the fourth day following this inoculation all leaves rubbed with water, with juice containing the virus of cucumber mosaic, or with juice containing the virus of ring spot, were observed bearing numerous necrotic spots typical of the primary lesions of aucuba mosaic. The leaves rubbed with juice containing the cucumber-mosaic virus or the ring-spot virus bore approximately the same number of lesions as those rubbed with water. Three of the 10 leaves rubbed with tobacco-mosaic virus bore no lesions whatever. The other 7 leaves bore a few scattered lesions on their edges. The experiment proves that no protection whatever is afforded against aucuba mosaic by the direct inoculation of leaves with the virus of either cucumber mosaic or ring spot under conditions favorable for the development of protection when tobacco mosaic is used.

During the course of further studies on the possibility of protecting leaves by inoculating them with these viruses, it was observed that lesions of ring spot retard the development of the primary lesions of aucuba mosaic. When a leaf bearing a small number of ring-spot lesions is inoculated by rubbing the upper surface with juice containing the virus of aucuba mosaic, lesions of the latter appear in the green portions of lesions of the former. Such lesions develop more slowly than those in other parts of the same leaf.

DISCUSSION

The experiments reported in this paper show that the leaves of *Nicotiana sylvestris* plants may be immunized against aucuba mosaic by inoculation with tobacco mosaic or its attenuated strains. They may also be immunized by inoculation with attenuated strains of aucuba mosaic. The very conspicuous primary lesions produced by aucuba mosaic give a convenient and accurate test that makes possible observations on the rate of development, extent, and specificity of the immune reaction.

The leaves of healthy *Nicotiana sylvestris* plants are highly susceptible to infection by the virus of aucuba mosaic. The inoculation of such plants with tobacco-mosaic virus does not immediately alter their susceptibility to aucuba mosaic. A definite period of time must elapse before plants inoculated with the virus of tobacco mosaic acquire immunity from aucuba mosaic. It is evident that immunity develops after, and as a result of, tobacco-mosaic infection. The infection of any given leaf by the virus of tobacco mosaic may result from direct inoculation of the leaf, or from invasion in the course of systemic infection. If infection is by direct inoculation, the areas immunized coincide very closely with the areas inoculated. If complete immunization is desired, the inoculation must be heavy. In that case the interval between inoculation and complete immunization is about 2 days. There is at this time no visible evidence of infection. It is a striking fact that tissues that are highly susceptible become immune within 2 days without undergoing any visible change. If

infection is by systemic invasion, the interval is somewhat longer. It is, however, complete regardless of the extent or degree of severity of inoculation. All leaves in which clearing-of-veins or mottling appears become immune. Leaves or parts of leaves too old to be visibly affected by systemic invasion, acquire immunity very slowly. Even in young plants, such leaves remain susceptible for a month or 6 weeks after the inoculation of the plants. They finally become immune without losing their deep green color. These leaves, however, may be quickly immunized at any time by direct inoculation. The virus of aucuba mosaic does not multiply appreciably in protected cells. This undoubtedly accounts for the failure of lesions to develop in protected areas.

Nothing whatever is known regarding the nature of the process by which leaf tissues are protected against aucuba mosaic. The immune reaction is so closely associated with the spread and multiplication of the virus of tobacco mosaic that it has not been possible to determine whether the inhibiting factor may be the virus or some substance produced in its presence. It is conceivable that closely related viruses multiply in and occupy the same parts of infected cells and that, when these parts are filled by one of them, there is no room for another. It is also conceivable that related viruses make use of the same materials in their multiplication, and that, when the materials are exhausted by one, the multiplication of another is prevented. On the other hand, it is possible that protection may be due to immunizing substances produced by infected cells. It does not seem likely that protection results from morphological changes in the tissues, since immunity is acquired by old as well as by young tissues. The deep green areas of leaves mottled by tobacco mosaic contain less virus than the light green areas. They are, nevertheless, immune from aucuba mosaic. Whatever the factor may be that protects against aucuba mosaic, it doubtless operates in limiting the severity of tobacco mosaic. The fact that this disease does not, under ordinary conditions, kill plants, may be due to a defense mechanism which holds the multiplication of the virus in check. There is at present no satisfactory technique for demonstrating such a mechanism for the virus of tobacco mosaic.

There is evidence that the immunity conferred by tobacco-mosaic virus and its attenuated strains may be specific. Cucumber mosaic gives no protection against aucuba mosaic. Plants that have recovered and are immune from ring-spot are quite susceptible to aucuba mosaic. Much further work must, however, be done before the specific nature of the immune reaction is proved. If the reaction is found to be specific, it will be useful in differentiating related from unrelated viruses.

The immunization of plants against aucuba and tobacco mosaic by means of highly attenuated strains of the latter offers interesting possibilities for the practical control of these diseases. Some attenuated strains

cause such slight injury that their use in the protection of plants might be advisable in situations where the incidence of one or both of these diseases is high. The principles involved are the same as those made use of in human and animal pathology for immunization by vaccination.

SUMMARY

Certain symptoms by which tobacco and aucuba mosaics may be distinguished on *Nicotiana sylvestris* are described.

Cross-immunity studies show that plants infected by attenuated strains of the virus of aucuba mosaic become immune from unattenuated virus. Plants infected by tobacco-mosaic virus become immune from aucuba mosaic, except in the youngest leaves.

Mature leaves of healthy plants inoculated with tobacco-mosaic virus acquire immunity from aucuba mosaic in the parts inoculated.

Leaves must be heavily inoculated if complete immunization is desired. The immune reaction is closely confined to the parts inoculated.

Immunity resulting from systemic infection is closely associated with invasion of tissues by the virus of tobacco mosaic.

Immunity resulting from direct inoculation develops within 2 days. That following systemic infection develops more slowly.

The virus of aucuba mosaic multiplies little or none in protected tissues.

Immunity resulting from direct inoculation develops in old as well as in young plants.

Protection by local inoculation is not confined to cells actually inoculated.

Plants inoculated with attenuated strains of the virus of tobacco mosaic become immune from both tobacco and aucuba mosaics.

Due, it is believed, to inadequate technique, reciprocal cross-immunity tests failed to demonstrate immunization by aucuba mosaic.

No protection against aucuba mosaic is afforded by the infection of plants either with cucumber mosaic or with ring spot of tobacco.

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STUNT—A VIROSIS OF DELPHINIUM[1]

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INTRODUCTION

The garden varieties of perennial delphinium in the state of Washington are affected with a virosis, which is readily transmissible by mechanical means to tomato, cucumber, tobacco, and several other species of plants.

A virosis of delphinium closely resembling ring spot of tobacco was reported in 1928 by Valleau.[3] The same year Linford[4] reported yellows of delphinium in Utah. The characteristic symptoms of yellows caused the plants to become chlorotic in advance of blossoming followed by a leafy proliferation of the floral parts. In 1930 Valleau[5] reported a virosis of delphinium, transferable to tobacco, which appeared to be identical with the "coarse etch" of tobacco.

In a more detailed report by Valleau[6] in 1932 he states "The Delphinium virus corresponds most closely to the typical cucumber viruses, ..."

A disease of delphinium designated as witches' broom by Hungerford[7] was reported in Idaho in 1933. He stated ". . . The appearance and general conditions surrounding the occurrence of this disease would indicate that it is probably due to a virus."

MATERIALS AND METHODS

The experimental work was carried on in the greenhouse and in the field at the State College of Washington, Pullman, Washington, in 1932 and 1933.

In October, 1932, a number of delphinium plants which appeared to be diseased, as well as some healthy plants, were transplanted into deep boxes in the greenhouse. These plants produced sufficient new foliage within 3

[1] Published as Scientific Paper No. 264, College of Agriculture and Experiment Station, State College of Washington, Pullman, Washington.

[2] The writer wishes to acknowledge the valuable assistance, advice, and criticism given by Dr. L. K. Jones and Dr. F. D. Heald during the progress of this investigation.

[3] Valleau, W. D. Ring spot. U. S. Dept. Agr. Bur. Pl. Indus. Pl. Dis. Rptr. Sup. 65: 419. 1927.

[4] Linford, M. B. Yellows. Pl. Dis. Rptr. Sup. 65: 420. 1927.

[5] Valleau, W. D. A virus disease of Delphinium reported from Kentucky. Pl. Dis. Rptr. 14: 118. 1930.

[6] ———. A virus disease of Delphinium and tobacco. Ky. Agr. Exp. Sta. Res. Bul. 327: 81–88. 1932.

[7] Hungerford, C. W. A new virus disease of Delphinium in Idaho. Pl. Dis. Rptr. 17: 5. 1933.

weeks to serve as a source of inoculum. When seedlings of delphinium, tobacco, tomato, cucumber, and several other species of plants were inoculated with the macerated leaf tissue[8] of the naturally infected delphinium plants a virosis became evident on the inoculated plants. Depending on the species of plants inoculated, symptoms became evident in about 4 to 20 days. The virus was recovered on tobacco plants from some of the inoculated species that failed to produce evident symptoms.

All of the species tested and recorded in this report were grown from seed and there was no evidence of seed transmission. The test plants were grown in the greenhouse at temperatures ranging from 65° to 75° F. Most of the plants under observation were grown in 3-inch pots but some that were held to maturity were transplanted to raised benches.

TRANSMISSION OF THE DELPHINIUM VIRUS TO DELPHINIUM AND OTHER SUSCEPTIBLE SPECIES

A virus naturally occurring on delphinium in the State of Washington has been experimentally transmitted by mechanical means to 4 varieties of delphinium and 12 other species of plants, including tomato, cucumber, and tobacco (Table 2). The virus was recovered from 12 of the 22 delphinium plants tested (Table 1). Sixteen other species of plants showed no evident symptoms when inoculated with the virus and the virus was not recovered from these plants when transfers were made back to tobacco plants.

The transmission of this virus has been demonstrated by evident symptoms produced on these inoculated hosts and by symptoms produced when inoculations were made on tobacco plants.

TABLE 1.—*Recovery of the virus on tobacco from delphinium naturally infected in the field*

Number of delphinium tested	Number positive	Inoculations to tobacco	
		Number plants inoculated	Number positive
22	12	145	51

SYMPTOMS ON DELPHINIUM

The symptoms attributed to this disease on naturally infected delphinium in the garden or nurseries are extremely variable. One of the outstanding symptoms is a stunting and dwarfing (Fig. 1, B) and eventual death of

[8] Jones, L. K. A new method of inoculating with viruses. (Abst.) Phytopath. 22: 998–999. 1932.

TABLE 2.—*Results of inoculations to various species of plants with delphinium virus*

Species of plants inoculated	Number inoculated	Number positive	Inoculations back to tobacco	
			Number inoculated	Number positive
Delphinium sp.				
Chimes Blue	15	5	115	19
White Butterfly	15	9	115	29
Wrexham	10	1	70	9
Common Belladonna type	15	8	115	38
Nicotiana tabacum	53	51		
Lycopersicum esculentum	63	52	64	51
Nicandra physalodes	19	19	25	25
Solanum nigrum	12	5	6	4
Stellaria media	11	8	10	10
Lactuca scariola	5	3	17	11
Cucumis sativus (cucumber)	12	7	3	3
Zinnia sp.	5	2	2	2
Marrubium vulgare	4	2	7	5
Anthemis cotula	3	1	13	6
Capsella bursa-pastoris	5	3	13	13
Petunia hybridum	5	?	2	1
Solanum tuberosum				
Early Rose—virus free	4	0	30	0
Seedling—virus free	19	0	65	0
Datura stramonium	24	0	68	0
Solanum pseudocapsicum	5	0	5	0
Chenopodium album	6	0	17	0
Pisum sativum				
Canner's Champion	12	0	24	0
Gradus	12	0	24	0
Capsicum annuum	5	0	5	0
Solanum melongena	5	0	2	0
Raphanus sativus	5	0	5	0
Viola tricolor (pansy)	5	0	5	0
Aster sp. (aster)	5	0	5	0
Spinacia oleracea	1	0	2	0
Lactuca sativa	5	0	10	0
Althaea rosea	1	0	2	0
Malva rotundifolia	2	0	5	0
Lamium amplexicaule	1	0	3	0
Amaranthus retroflexus	2	0	4	0

the plant. From general observations it appears that the stunting and dwarfing are progressive from season to season.

Because of this characteristic stunting and decline of delphinium affected with this virus, an appropriate name for the disease is "Stunt," which is here proposed. Infected delphinium plants grown in the open were slower in starting growth in the spring than healthy plants. Some plants showed extreme dwarfing with a chlorotic appearance. The foliage of these plants was extremely dwarfed and mottled or chlorotic with savoying and cupping of the laminae in varying degrees (Fig. 2, B).

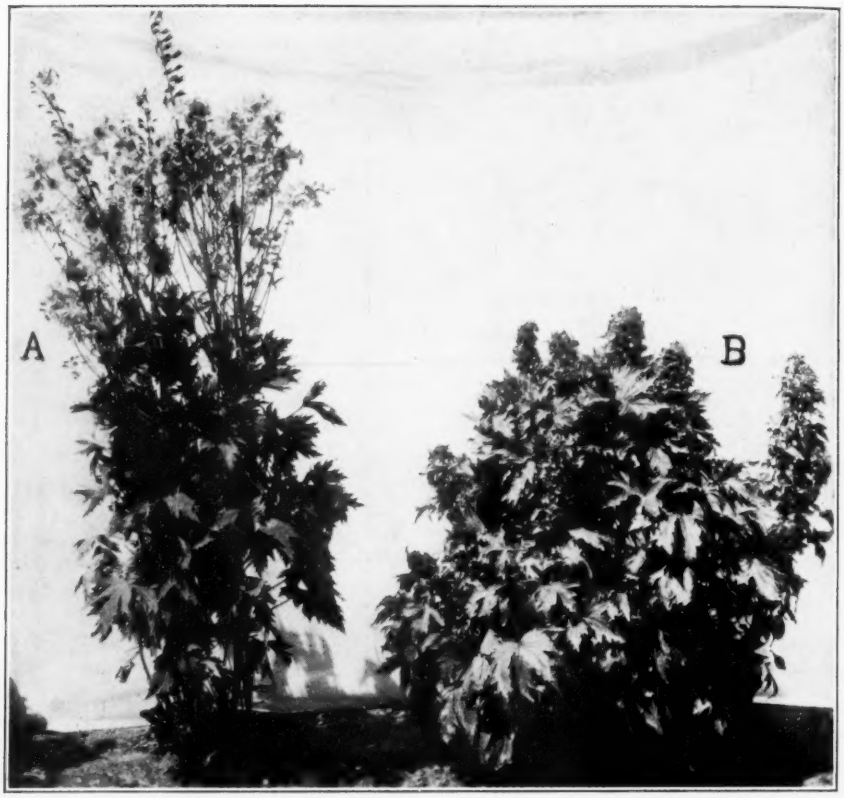

FIG. 1. A. Healthy delphinium. B. Dwarfing and floral proliferation due to natural infection of the virus.

The chlorosis was confined to the margins of the leaves. In addition, dark brown to tarry black necrotic specks and streaks of variable sizes and shapes up to 5 mm. or more were often evident on the leaves (Fig. 2, C). When these brown, necrotic, dead areas were confined to a single marginal spot the leaf became distorted due to the uneven marginal growth (Fig. 2, C). In many cases these necrotic areas would coalesce and involve entire leaves and thereby produce complete killing. Leaf petioles and stems often exhibited similar necrotic spots and streaks. It was not uncommon to find tarry black lesions 2 to 3 inches in length completely girdling the stems and petioles and thereby causing death of the affected shoots. New shoots arising from such devitalized plants were more numerous and materially stunted. All of the above symptoms were evident on the first spring growth and continued until about the first of June. Severely infected plants on that date were only 6 inches tall in contrast to 2 feet or more in height for

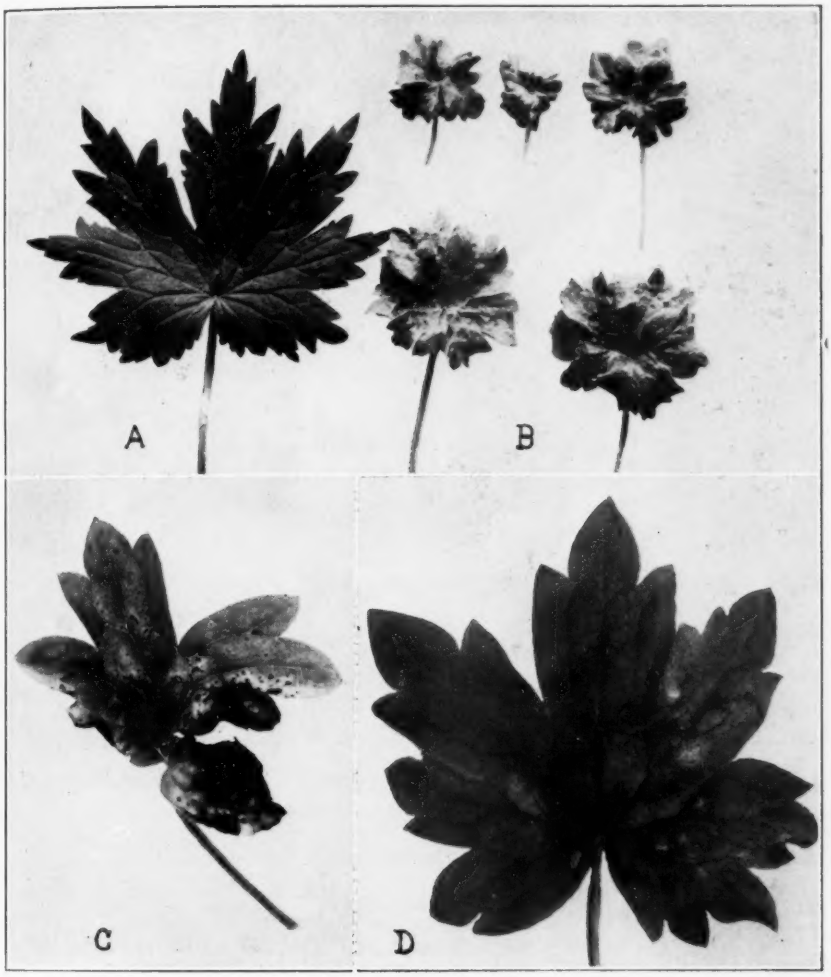

FIG. 2. A. Normal leaf of delphinium. B. Foliage with dwarfing, mottle, chlorosis, savoying, and cupping of the laminae. Field occurrence. C. Tarry black necrosis of the leaf, natural infection. D. Tarry black ring and line pattern of the leaf as a result of artificial inoculation in the greenhouse.

healthy plants or those exhibiting slight symptoms. Later in the summer there was an excessive proliferation of the flowering stalks, which produced a bunchy witches' broom appearance (Fig. 1, B). The flowering parts failed to develop normally but produced a characteristic leafy proliferation with varying degrees of transformation from slightly greenish flowers to pale green leafy structures (Fig. 3). Some of the severely infected plants in a plot grown by the horticultural department died early

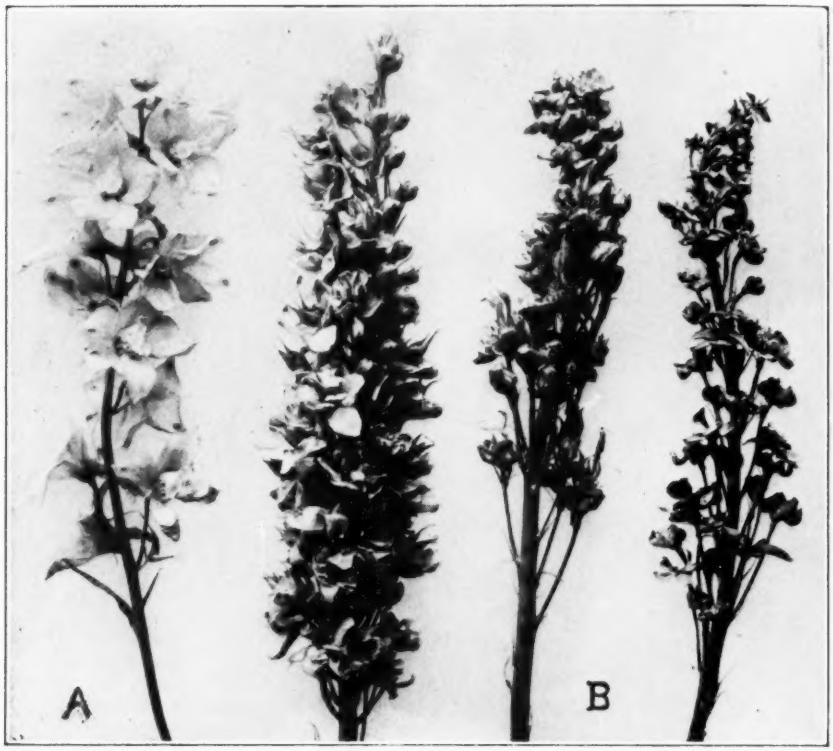

FIG. 3. A. Healthy inflorescence. B. Leafy proliferation of varying degrees of the floral parts. Field occurrence.

in the fall, following the production of the aborted floral parts. When such plants were examined it was found that the roots had so decayed and disintegrated that the stalks could be easily pulled or lifted out of the soil. The symptoms as described above were evident on plants grown under normal conditions. Because seedlings of delphinium, inoculated with the virus (recovered from delphinium) have not yet matured in the open, it cannot be stated definitely that the transformation of the floral parts to leafy structures or the premature dying of the plants in the fall is definitely associated with the virus. Inoculated delphinium seedlings grown in the greenhouse have exhibited the other symptoms that are common to plants grown in the open.

The virus, which has been recovered on tobacco, when transferred to six-months-old delphinium seedlings, during October, produced a slight yellowing of the foliage and a dark brown necrotic ring and line pattern (ring spot type) on the inoculated leaves (Fig. 2, D). Streaks or blotches of brownish black necrotic areas soon appeared on the petioles. The following

spring the characteristic tarry black necrotic spots appeared on the new foliage.

Plants exhibiting this disease have been reported from various sections of the State. One nurseryman stated that it was quite common in his plantings and that he had received complaints from various customers in different parts of the United States that some plants received from him failed to produce satisfactory growth. From this and other available information it would appear that the disease is quite generally distributed throughout the United States. There is some evidence that it has been present in this State for at least 15 years.

SYMPTOMS ON TOBACCO

Inoculations were made to *Nicotiana tabacum* (Connecticut Havana) plants in October, 1932, from affected delphinium that had been transplanted into boxes in the greenhouse. The delphiniums produced sufficient foliage from which to secure inoculum. With the first transfer from delphinium to tobacco, symptoms may not appear until about the 15th to 20th day, but subinoculations to tobacco more often produced symtoms in 4 to 6 days. The symptoms on tobacco are variable, sometimes showing as a white, necrotic etching, with ring-and-line pattern of variable forms, while in other cases the first recognizable symptom is a vein clearing (Fig. 4, B). Some plants never produce the ring-and-line pattern but exhibit a vein clearing that later changes to a blotchy, irregular, usually interveinal mottle which may coalesce to form extensive blotchy chlorotic areas (Fig. 4, A). In more mature plants these blotches may involve the major part of the older leaves. In addition the tobacco leaves usually are somewhat ruffled, producing a wavy and crinkled appearance. Some leaves may appear normal but subsequently produce a faint mottle which may or may not develop into the blotchy type. The necrotic ring and line patterns are confined to the first few leaves, while the older plants seldom show evidence of this symptom but rather exhibit the blotchy type of foliage. In most plants inoculated with this virus, dwarfing is usually very evident.

This virus, when combined with tobacco mosaic, appears to intensify the symptom expression of the tobacco mosaic on tobacco. The combined viruses sometimes produce symptoms comparable to white mosaic of tobacco, and also, sometimes, cause some vein or leaf necrosis. The delphinium virus, when combined with the latent virus of potato, generally produced a type of spot necrosis similar to that produced by the combination of veinbanding and the latent virus. In a similar manner the delphinium virus and veinbanding produced a type of spot necrosis similar to the above combination. From this it appears that the delphinium virus is variable

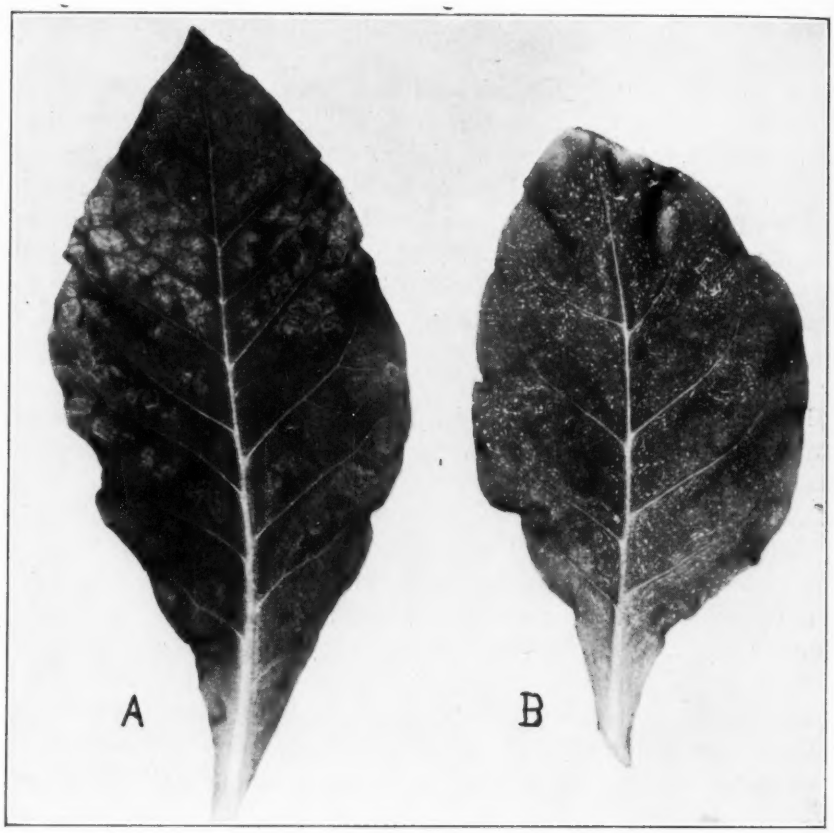

FIG. 4. Havana Connecticut tobacco inoculated with the delphinium virus. A. Blotchy, irregular mottle. B. White necrotic etching with ring-and-line pattern.

and capable of producing variable symptoms when combined with other specific viruses.

SYMPTOMS ON TOMATO

The delphinium virus is readily transferred to tomato, producing characteristic symptoms. The plants at the end of 12 to 15 days usually are dwarfed when compared with healthy checks. The first symptom, other than dwarfing, is a definite vein clearing, evident between 15 and 20 days after inoculation. This is followed within a few days by a definite downward rolling of the leaflet margins, a hooking down of the ends of the leaflets as well as the apical part of the leaf and the leaflets often become deeply lobed or cut (Fig. 5). The foliage at this stage shows a faint but perceptible mottle, which intensifies with the age of the plant. The interveinal tissue becomes lighter colored in contrast to the

FIG. 5. John Baer tomato exhibiting deeply lobed leaves and curling and distortion of leaves and stems as a result of inoculation with the delphinium virus.

darker areas bordering the veins. As this mottling increases there appear irregular large blotchy areas on the leaves similar to those described on tobacco. This blotchy type of mottling is quite characteristic also on several of the susceptible hosts reported below. Plants infected with this virus usually exhibit a pale green color, and there is a slight to moderate savoying of the leaves. The fruit, however, evidently is not affected. These symptoms are soon followed by further distortion of the leaves in which they become curled in a corkscrew fashion, turning on their axis as much as 180° or more. This curling also occurs on the main stem or on lateral branches (Fig. 5).

Combination on tomato. The delphinium virus in combination with tobacco mosaic produced on the tomato extreme and variable symptoms. In addition to the symptoms resulting from either virus alone there appeared definite filiform leaves (Fig. 6) and some plants exhibited a type of streak (Fig. 7), somewhat resembling those produced by the latent virus and tobacco mosaic. The former combination caused dwarfing, necrosis of the leaves, and some petiole streak, but there was less apical killing, stem and petiole streak, or the greasy brown lesions on the fruit that is common with the latter combination.

FIG. 6. Filiform leaf of tomato produced by the combination of the delphinium virus and tobacco mosaic.

The delphinium virus, when combined with the latent virus of potato, gave, in addition to the symptoms produced by either virus alone, a type of spot necrosis and some killing of the lower foliage. It caused also a type of mottle closely resembling mild symptoms of tobacco mosaic.

The combination of the delphinium virus and veinbanding failed to give additional symptoms to those produced by these viruses when found alone on tomato.

SYMPTOMS ON OTHER HOSTS

Apple of Peru, Nicandra physalodes. The first evident symptom appearing in 4 or 5 days consists of water-soaked areas of variable size.

FIG. 7. A type of streak of tomato produced by the combination of the delphinium virus and tobacco mosaic.

These soon coalesce, resulting in large irregular brown necrotic lesions that may involve the entire leaf and cause it to die and fall from the plant (Fig. 8, C) with the possible resultant death of young plants. In any event the plant is materially dwarfed; but, if not killed, it will make a slow

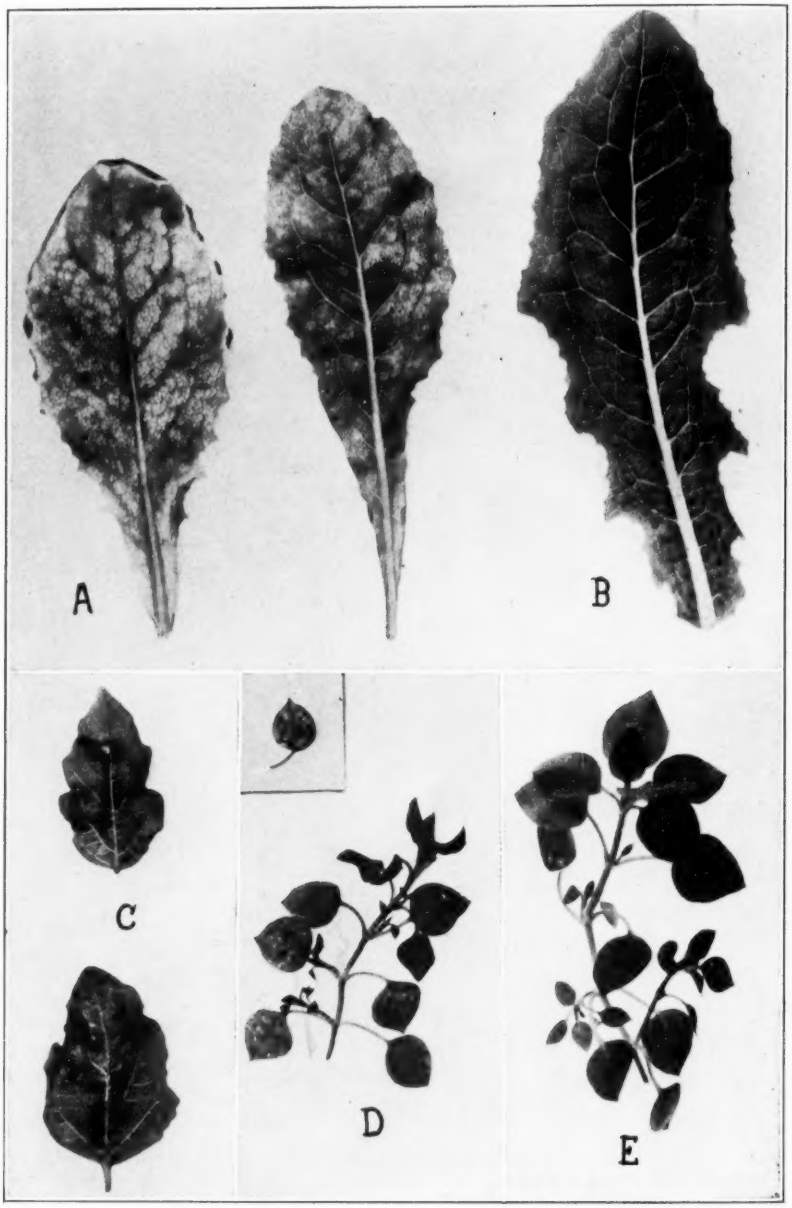

FIG. 8. A. Dwarfing and blotchy mottle on *Lactuca scariola* produced by artificial inoculation with the delphinium virus. B. Normal leaf of *L. scariola*. C. Brown necrotic lesions produced on *Nicandra physalodes* by the delphinium virus. D. Mottle and folding of leaves of *Stellaria media* produced by the delphinium virus. E. Normal stem and leaves of *Stellaria media*.

growth. The new foliage often may fail to produce evident symptoms; but the virus is systemic, since it can be recovered from foilage that fails to exhibit symptoms.

Black Nightshade, Solanum nigrum. The symptoms produced on black nightshade when inoculated with the dephinium virus closely resemble those on this species when inoculated with tobacco mosaic. The most common symptom is a mottling of light and dark green areas. There is no appreciable savoying of the foliage with either tobacco mosaic or delphinium virus, but with the latter there is a more conspicuous irregular blotchy type of mottle, which corresponds to the blotchy mottling on tomato and tobacco plants. This host appeared to be less susceptible than tobacco or tomato.

Chickweed, Stellaria media. When chickweed was inoculated with the delphinium virus it became dwarfed and produced a mottle with chlorotic interveinal tissue. The veins usually retained their green color but, in some cases, they also became chlorotic (Fig. 8, D). The apical leaves usually became completely folded over, with the midrib as the central point of the fold (Fig. 8, D).

Prickly Lettuce, Lactuca scariola. The symptoms on prickly lettuce consist of dwarfing, a definite curling and folding of the leaf with a blotchy mottle which is produced by numerous small, white, necrotic areas (Fig. 8, A).

Cucumber, Cucumis sativus. The more pronounced symptoms on cucumber foliage consist of a mosaic mottle and rigidity of the foliage similar to that produced by true cucumber mosaic. Often, there is also a sharp downward or an upward bending of the distal portion of the leaf. This turning downward or upward may be slight or it may form an angle of 90°. Some fruit which matured failed to show evident symptoms.

Zinnia, Z. elegans. The more evident symptoms on zinnia consist of dwarfing, excessive crinkling, and rigidity of the foliage. There was no apparent mottle evident.

Horehound, Marrubium vulgare. The only evident symptom on horehound is a general dwarfing.

Other Susceptible Species of Plants. Symptoms were not evident on dog fennel, *Anthemis cotula,* shepherd's purse, *Capsella bursa-pastoris,* or petunia, *Petunia hybridum,* when inoculated with the delphinium virus, but the virus was recovered from each when subinoculations were made to tobacco plants (Table 2). In each case a young leaf, above the point of inoculation, was taken as a source of inoculum from these plants about 3 weeks following the original inoculation.

Nonsusceptible Species of Plants. Several unsuccessful attempts were made to recover from the following 16 plants the delphinium virus with

which they were inoculated: *Solanum tuberosum* (virus-free Early Rose and seedling stock), *Datura stramonium, Solanum pseudocapsicum, Chenopodium album, Pisum sativum* (Canner's Champion and Gradus varieties), *Capsicum annuum* (pepper), *Solanum melongena* (egg-plant), *Raphanus sativus* (radish), *Viola tricolor* (pansy), *Aster* sp. (aster), *Spinacia oleracea* (spinach), *Lactuca sativa* (lettuce), *Althaea rosea* (hollyhock), *Malva rotundifolia* (mallow), *Lamium amplexicaule*, and *Amaranthus retroflexus*.

DISCUSSION

It has been shown by other investigators that delphiniums in a few sections of the United States are affected with a virosis and that other diseases not definitely proved are presumed to be of virus origin. The virus that has been used in this study is capable of producing extremely variable symptoms on delphinium and some other plants. For this reason it is not at all improbable that some or all of the symptoms may be expressed under different environmental conditions. From this point of view it may be assumed that a single virus is responsible for these variable symptom expressions. Delphinium, inoculated with this virus, produced such symptoms as ring spot, necrosis, and chlorosis similar to those reported by other authors. Plants from which the virus was secured exhibited such symptoms as necrosis, chlorosis or yellowing, a witches'-broom effect, and a reversion of the floral parts to leafy structures.

The delphinium virus appears to be capable of causing a different response when combined with other viruses. When combined with the latent virus of potato, it produced a type of spot necrosis similar to that resulting from the combination of the veinbanding virus and the latent virus. Similarly, it caused a type of spot necrosis on tobacco when combined with the veinbanding virus. It is evident that the delphinium virus can be substituted for either the veinbanding or the latent virus to produce spot necrosis on tobacco plants. Again, when combined with tobacco mosaic, it responds much the same as the latent virus when in combination with tobacco mosaic. This virus, plus tobacco mosaic, caused a type of streak on tomato and leaf necrosis of tobacco similar to those produced by a combination of tobacco mosaic and the latent virus. Again, it is capable of producing fern leaf of tomato when combined with tobacco mosaic and therein resembles the reaction of cucumber mosaic on tomato. The fern leaf is not caused by the delphinium virus, alone. It would then appear that this virus has two rather peculiar properties in that it is capable of producing such variable symptoms on many different species of plants and that it is variable in its response when combined with other viruses.

SUMMARY

The virosis of perennial delphinium known as "stunt" or witches' broom is common in Washington, Idaho, and other portions of the United States and causes a dwarfing, an increase in the number of shoots, various foliage symptoms, including dwarfing, mottling, chlorosis, savoying or cupping of the laminae, necrotic patterns on both stems and leaves, and greening and malformation of floral parts.

The virus has been transmitted by mechanical methods to tobacco, tomato, cucumber, apple of Peru, black nightshade, chickweed, prickly lettuce, zinnia, horehound, dog fennel, shepherd's purse, and petunia, causing visible responses of various types on all except the last 3, on which it appeared to be latent, as determined by reinoculations into tobacco.

The virus caused no visible effects and also was not latent on 16 other species of cultivated and wild plants, as determined by inoculations on tobacco. No other suscepts have been known to contract the disease under natural garden conditions.

The delphinium virus, when combined with tobacco mosaic, intensifies the symptom expression on tobacco, causes variable symptoms on tomato, including string-like or filiform leaves and a type of streak; when combined with the potato latent virus on tomato, symptoms not shown by either virus alone were exhibited.

The vectors of the delphinium virus have not yet been determined.

STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON.

VITALITY AND VITALITY DETERMINATION IN POTATOES[1]

GEH. REG.-RAT PROF. DR. OTTO APPEL

(Accepted for publication July 15, 1933)

When, at the beginning of this century, the diseases of potatoes became increasingly more frequent, with the resultant unfavorable effect on the crops, the agriculturists interested in potato culture became somewhat disturbed. Everywhere the leaf roll, curl, and mosaic and other causes of damage became particularly prevalent, in part, in disquieting proportions.

Since the scientific investigations into these various diseases—collectively called degenerative diseases—failed to bring quick results, and in as much as their eradication became an urgent necessity, ways and means of evaluating the seed potatoes were adopted; in other words, the state of health of the crops from which seedlings were to be taken was determined. This method of evaluation has spread from Germany all over the world. I myself was able to cooperate in 1914 in the development of appropriate measures in the United States.

In the meantime, however, it had become apparent that, even with tested seedlings, there was no absolute certainty of healthy crops. On the contrary, it can be noted more frequently that potatoes from an apparently healthy field produce defective crops the following year. This has led us to investigate the possibility of determining from the seed potato itself whether or not it will produce a healthy plant. Thus we came to formulate the concept of tuber vitality.

Conditions are more or less similar with grain. At first, one was guided by the determination of its germinability; but later, when one could see that this was not sufficient, one proceeded to the determination of the germinating power. One has determined, therefore, not only the presence of vitality but also the degree of vitality. A similar procedure was not possible for potatoes, since the germinating conditions of the potato do not indicate the state of its health. We, therefore, had to approach the subject from a different angle and examine the various conditions of chemical composition and of vital manifestations. We have succeeded to arrive, by two different routes, at useful results. I am particularly happy to be able to present these results to you today.

It is not my intention to discuss the various degenerative manifestations themselves, nor to detail the various theories and experimental results related to them. I shall rather report to you on the copper method of Bechhold and Erbe and the potentiometric determination of the seed quality

[1] Paper read by Dr. Appel at the meeting of The Am. Phytopath. Soc.—Chicago "Century of Progress" program, June 21, 1933.

according to Wartenberg and Hey. The former is the result of cooperation between the Biological Reichs Institute for Agriculture in Forestry in Berlin-Dahlem and the Institute for Colloidal Research in Frankfort a. Main, the latter originated in the Botanical Laboratory of the Biological Reichs Institute.

THE COPPER METHOD OF BECHHOLD AND ERBE

The copper method appears at first to be very simple. However, in spite of its simplicity it contains several very important sources of error. Therefore, it will be necessary for me to call attention also to these errors.

The preparation of the tubers for examination is quite simple. Take a strip of copper, 1.5 cm. wide and 10 cm. long and insert it lengthwise into the tuber so that a piece of copper projects from either end. The tuber thus prepared is then placed in a warm moist chamber, which is placed in a thermostat. The tuber remains here for 8 hours at 38° C. Then the moist chamber is taken out of the thermostat and is allowed to stand for 16 hours in a room at room temperature, *i.e.*, at 18 to 20° C. Thereupon the copper strip is removed from the potato and the tuber is cut in two. The cut should be made in the direction of the insertion but at right angles to the flat surface of the copper. On the cut surface, along the channel made by the copper plate, there appears a more or less intensely colored zone that, at first, is brownish or reddish but assumes, upon exposure to the air, a dark gray or black tone. The extent of the discoloration is characteristic for the degree of health or degeneration of the tuber. A degenerated tuber shows none or only a trace of discoloration, while a healthy tuber shows, in typical cases, a broad zone of color, which is of a dark tint around the point of insertion.

However simple the operation of this method might appear at first glance, the evaluation of the results is difficult: because, as mentioned above, there exists a series of sources of error that are difficult to control and that may influence the results. First of all attention must be paid to the exact maintenance of the temperature of 38° C. in the thermostat. This temperature must be positively obtained and maintained throughout, otherwise comparative evaluations of cropping values are impossible with this method. The principal difficulty lies in the accurate evaluation of the discolored zones; not so much because such an evaluation is difficult because of the different picture presented by each cut surface as a result of the irregular outline of the edge of the zone, but rather because it is certain, even today, that the extent of the discolored zones in some cases is affected not only by the degree of degeneration, but also by the variety and other specific considerations of the origin of the seed. Thus, for instance, there are varieties whose discoloration in cases of vitality are smaller or larger than that of

other varieties. It also happens that, within one and the same variety, seed from one origin shows no discoloration—because of influences yet unknown—and thus presents the picture of a degenerated potato, while later on in cultivation a perfectly vital growth is obtained. The reverse case, where a degenerated tuber presents the picture of a healthy one, is very rare and has not yet been positively demonstrated.

At present investigations are being undertaken for the purpose of eliminating these sources of error in order to make the method practical for the farmers. Unfortunately, the specific method of examination and the impossibility of looking at the interior of the tuber and the changes that are taking place within it, make it difficult to recognize the sources of error. It is hoped, however, that in the near future the copper method of Bechhold will become a reliable method for the practical agriculturist.

Nothing can yet be said concerning the details of the copper method. It is the result of accidental observation and we do not yet know today why discoloration occurs in a healthy tuber and why the inside of a diseased tuber does not discolor. Just as little do we know about the rôle played in the whole process by the copper strip. All that has thus far been found in this respect must be designated as uncertain experimental results. These, however, already show that we are confronted with the most difficult problems of physiology. The copper method, as well as another that I shall explain to you later, are the practical achievements of scientific experiments that may be put to practical use within a reasonably short time from now. Furthermore, they also point out for us the way to a new type of experimentation that will perhaps bring us a great deal nearer to the solution of the problems of potato degeneration.

WARTENBERG AND HEY'S POTENTIOMETRIC METHOD OF EVALUATION OF CROPPING VALUES

Every practical method requires mastery over sources of error. In this respect Bechhold's copper method is outdistanced by far by the potentiometric method of evaluation of cropping values elaborated in the Botanical Laboratory of the Biological Reichs Institute by Wartenberg and Hey. This method can boast today of extensive investigation and a considerable series of successful evaluations for practical purposes.

The preparation and operation of the measurements are not so simple, and a great deal more experience is required for the evaluation of the individual tests than is necessary with the copper method. On the other hand, however, the results obtained by Wartenberg and Hey's method have proved so far to be considerably more reliable and give, with a sufficient number of individual determinations, not only a definite average value for the degree of degeneration of a certain variety but also an irreproachable

picture of the percentage distribution of healthy and diseased tubers within one and the same variety.

The seed potatoes that are to be examined as to their cropping value are each separately washed, peeled, and grated to a mash on a glass grater. The mash of each tuber is put into a beaker and mixed with double-distilled water in the proportion of about 1 to 2. After stirring, the froth is removed from the mash by means of a glass spoon. A clean platinum electrode is then inserted into the center of the settled mash in each glass. The platinum electrode should first be cleaned with sulphuric acid and well rinsed with tap water and distilled water. The above arrangement represents a half element, since the platinum of the electrode receives an electrical charge in the mash, and in the course of time there develops on the platinum sheet a definite electric potential.

Such a half element may be compared to $\frac{1}{2}$ of a pocket battery. Therefore, if it is being connected with another half element, converting it into a complete element or a so-called double half element, it is possible to obtain an electric current from this arrangement. If a second half element, one with a known constant potential, is taken, it is then possible to measure the difference of potential of the double-half element and to calculate the electric charge which the platinum sheet has received in the potato mash.

In the potentiometric method of evaluating cropping value according to Wartenberg and Hey, such a double half-element arrangement is employed and the difference of potential of the two element poles measured. As a parallel half element for purposes of comparison, the saturated calomel electrode, which always has a constant potential, is employed. It is connected on one side by a KCl-electrolyte bridge with the mash of the potato half element, and on the other side the current between the 2 metallic poles is connected by means of a wire connection with the measuring apparatus. As measuring apparatus serves the Trenel Potentiometer by means of which the difference of potential of the 2 half elements is measured according to Poggendorf's Compensation Method. We have constructed recently in Dahlem a measuring apparatus with which one person is able to measure 40 tests simultaneously.

The time element in the determination of each test can be divided into 2 parts. The first part comprises the duration of the initial curve, that is, the time in which the potential of the electrode adjusts itself to the constant value; and the second part serves as a control of the constant value. The value obtained at the beginning of every individual measurement is arbitrary and has no relation to the degree of degeneration of the potato tuber, which has been utilized to make the potato half element. The latter, at the very beginning of the test, may represent either the positive or the negative pole of the double half element. If, however, the readings are repeated in

intervals of about 10 minutes, it will be noted that during the readings the difference of potential between the potato half element and the control electrode—the calomel electrode—changes. The potato half element in the course of time becomes more and more negative in relation to the calomel electrode. Finally, the change decreases and then the difference of potential remains constant. This is the end value towards which the initial curve is aiming. The end value is positively ascertained by means of 2 to 3 hours of control of this value. It must not change any more during this period.

As mentioned above, the initial reading of a test bears no relation to the degree of degeneration of the tuber. This initial value together with the entire first part of the readings represents a necessary evil in this method that it has yet been impossible to circumvent.

The question may be asked why the end value is reached only after a curve has been established, sometimes over several hours. We have studied this question in the course of special investigations because it is of the greatest importance for the accuracy of the method. These investigations have not yet been completed, but they have already shown that the results are highly interesting from a theoretical viewpoint and that they satisfy the practical requirements; in other words, the theoretical basis of this method is firmly established.

With regard to the practical operation of the method, it may be stated that the end value depends upon the beginning and the general course of the initial curve. The 2 latter points are determined by the preparation of the electrode, the sensitivity of the measuring apparatus, the specific characteristics of the potato tuber, which, however, do not affect the end value, and most of all by the indefinable ability of the person who is using the measuring apparatus.

A description of the experiments, however interesting they might be theoretically, would be going too far. I have the task to report on practical matters and shall, therefore, limit myself to a description of those measures that have a practical significance for this method.

One of the reasons for the initial curve is the original charge of the electrode, which depends upon the character of the cleaning material as well as upon the length of time during which the electrode has been in contact with the air. In our own case, the original charge is normally always positive. The change in the charge of the electrode in the potato mash does not occur suddenly, but requires a certain time expressed in the gradual change of the measured difference of potential.

Another reason for the initial curve is the polarization of the electrode. This is explained by the fact that, at the beginning of the test, one does not know in which range of potential the end value will lie. Therefore, one

establishes arbitrarily a compensative resistance and thereby, if it is too small, sends an electric current to the electrode that is then electrically charged. The effect of polarization is similar to that of the original charge; some time is then required until the action of the potato mash on the charge of the electrode becomes manifest.

A third reason for the initial curve is to be sought in the potato mash itself. The electric charge, which has been communicated to the platinum electrode by the potato mash, is tied up in the latter with a substance that is unevenly distributed in the tissue suspension shortly after its preparation. There must be first a neutralization of concentration so that this substance becomes present in the fluid of the mash in the same concentration as in the suspended tissue particles. Until this has happened, the constant potential cannot be attained. The neutralization of concentration, the diffusion of a substance from the intact cells of the tissue particles into the diluent, is a process that requires a certain time. The establishment of the potential is a definite function of time, simply because the substance that diffuses from the cells influences the potential, or rather the formation of the potential in a definite direction. It is definitely established only when a difference of diffusion of the substance from the cell to the surrounding fluid no longer exists.

The first and the third of the above described reasons for the initial curve may be methodically eliminated merely by postponing the readings until the following day. Immediately after the preparation of the potato half-element, the mash together with the electrode in it, are left overnight in an ice box at a temperature of about 10° C. The initial charge of the electrode is no longer present in the morning, and the neutralization of concentration is then complete. Then, under these circumstances, one still obtains an initial curve which, however, depends solely upon the intensity of the effect of polarization. If the readings are begun after establishment of the compensative resistances, which lie near the range of potential of the expected constant, polarization is unimportant and the aim of the measurement is soon reached. In this manner we have reduced the duration of a test from 10 to 4 hours, in which are included the 2 to 3 hours of control of the constant potential. I am anxious to emphasize once more that the half-elements must be positively kept in an ice box at the required temperature until they are used for the test. Higher temperatures might, it is true, hasten the neutralization of concentration, but they would have the drawback of hastening the fermentation of the tissue mash. If fermentation occurs, then the cropping value can no longer be determined. Through fermentation, one obtains potentials that lie in a different range from that of the potentials of healthy cropping values. Furthermore, the formation of potential through fermentation is characterized by the fact

that it does not lead to a constant; it gives, from reading to reading, progressively negative results.

On the other hand, it is not necessary to preserve the potato half-elements in the ice box at temperatures lower than 10° C. All that would be achieved would be to retard the neutralization of concentration.

The aim of every measurement is, as we have said above, only the constant value. If it has been obtained under proper conditions and if it has been controlled long enough, if it can be looked upon as definite, then the test may be terminated. The beginner should be certain to continue the control of the constant value for at least three hours. The constant value of the potential of the potato mash is in direct relation to the degree of degeneration of the potato. If it lies within a range of from −100 to −140 millivolts in relation to the calomel electrode, then we have before us, at any season and for any variety, healthy tubers, only, that will produce healthy plants in every case. Constant values that are more than −210 millivolts in relation to the calomel electrode always represent diseased plants in all cases. Those tests that become constant between 140 and 210 millivolts, however, should be evaluated according to the variety and the season in which the measurements have been made. I shall report in more detail later on the influences of variety and season.

First of all, I would like to demonstrate to you how Wartenberg and Hey have proved the relations between the measured values of constants and the degree of degeneration of the tubers. They sectioned the potato tubers by a cut from the crown to the stem, prepared one of the halves for measurements, and determined its value of constant, while they planted the other half in a greenhouse or in the experimental field where the growth of the plant from the tuber halves could be observed and compared with the measured cropping values. At present several thousands of such comparisons are available and have given good results. If the tubers of one or of different sources of seed potatoes of one or several varieties are planted in this manner in the field or in pots in sufficient numbers, according to their measured values, we note that, for example, with the variety "Erstling," which should be measured in January, healthy plants grow from the tuber halves of the first value range approximately up to the value of calomel electrode minus 195 millivolts. Then there follows another group within the next 15 to 20 millivolts approximately up to value 210, in which occur overlaps and in which the relations between potential values and degree of degeneration are not pronounced, where, therefore, healthy and diseased plants may fall within the same range. We speak here of a so-called *critical zone*. Below this critical zone, that is to say at values higher than −210 millivolts in relation to the calomel electrode, all plants are diseased. Up to the present it has not been possible to determine the type of disease

by means of the measured potential values. The relation between tuber potential and degree of degeneration, therefore, is not indicated in that, within a definite range of the constant values, only the leaf-roll disease is present, or in another range only the leaf-curl or mosaic is present, but merely indicates the difference between healthy and diseased tubers. The degenerative symptoms of leaf roll, curl, mosaic, bucket, dwarf, etc., appear to be primarily related to the variety and also to the source and origin of the seed. If the mean value of a certain source of seed lies lower in the disease range of the potential scale than that of another, the degree of degeneration is detectable also higher, that is, the entire vegetative potency is less under the same conditions of growth.

The outstanding advantage of the method I have just described to you lies in the fact that by means of this determination of vitality, all subjective errors can be excluded. On the other hand, the method has the drawback that the application of the test must be learned. The person undertaking such investigations must not only know the manner in which the test is managed, but must also be well acquainted with the errors that may arise. Furthermore, it is important to know how the values are to be judged during the different seasons and how one should deduce from a series of measurements a definite idea of the degree of the cropping value of tubers from a certain source.

In every investigation it happens that one or more measurements must be discarded because of possible errors. The fact that errors are easily recognized is another advantage of the method. The failures just mentioned may occur in different ways. The sources of error that may influence the individual measurements are divided into 2 classes. First, they may be errors having their origin in individualities of the measured tuber. The second group comprises errors that are traceable to outside influences. In all cases, the error manifests itself in an abnormal run of the curve, when the constant is either not attained at all, or else the curve ends in an apparently constant value, the position of which in the scale of potentials, however, is so improbably high or low that it falls outside the range of variation because of origin, and in this manner the error becomes visible at once.

The electrode failures are outstanding in the group of failures influenced by outside factors. New electrodes cannot be used for accurate measurements for a number of days. At times their potential differences in the same mash differ considerably from those of standard electrodes that have been in use for a long time. After the new electrodes have been used 8 or 10 times and have passed through a cleaning process, they give the correct value. One must therefore clean and test new electrodes until all of them

give nearly the same values in the same mash. The greatest permissible variation is 4 to 5 millivolts between electrodes with extreme values.

In order to make clear one failure against which one should be very much on guard, I shall now describe to you the type and form of the electrode. On a sheet of platinum, one square inch in size, is soldered a platinum wire, which passes through a glass tube and is connected at its upper end with a contact. The platinum sheet is fused partly into the lower end of the glass tube so that the upper fifth of its surface is covered with glass. The ordinary glass cannot be fused tightly enough on metallic platinum. Therefore, it is necessary to fix a piece of lead glass in the lower end of the glass tube into which the platinum sheet can be fused. In spite of these special measures, it may happen that cracks arise in the joined area, which often cannot be detected with the naked eye. The effect of such cracks manifests itself in potentials that are strongly displaced towards the positive side. This is due to the fact that the cleaning material enters the cracks during the cleaning process; it cannot be washed out and, later on, affects the formation of potentials. The influence is so great that it is noticeable in the course of the measurements. It produces, then, values that fall outside the normal range.

The position of the platinum sheet in the test beaker is of the greatest importance for exact measurements. One must make sure that it is inserted into the suspended tissue mash. It should not extend into the upper clear solution nor into the settled starchy layer at the bottom. If it does, the result would be a delayed curve, which, under certain conditions, does not become constant, or else the slow setting of the electrode may mislead the inexperienced observer into believing that a constant value has been obtained.

Fermentation, because of its causes, really belongs in the second group of errors, which I shall describe later. It manifests itself in a rapid fall of the potential that may reach from 300 to 400 millivolts below the lower border of the range of the potato potential. In most cases, the falling off on account of fermentation occurs only after hours of normal constancy. Certain tubers may show fermentation curves sooner than others. The reason lies in a yet unknown property of the tuber. In most cases, however, fermentation may be retarded and thus a sufficiently long constancy obtained by shortening the duration of the test by means of the above mentioned storage in an ice box.

More difficult than the recognition and elimination of the already described failures is, in many cases, the evaluation of those that arise from certain properties of the tubers. Particular attention is required with regard to the formation of melanine, the black discoloration, well-known to any one who has ever handled potato mash. The normal case in which

measurements may be made without difficulty is present when, before the beginning of the test, there is a clear distribution in the solution standing above the settled uncolored tissue. A zone of clear uncolored solution is present above the mashed tissue and a dark zone is sharply defined above this layer. This latter zone is dark brown to black, colored by colloidal melanine. The black zone of melanine formation does not occur in many varieties of seeds of different sources but remains a light to dark red. Since, also, there is here present a zone of colorless liquid between the mash and the layer of melanine, measurements are possible. Whenever the red color extends into the mash and, therefore, to the electrode, the test must be discarded. Likewise, all those tests must be discarded that show no or only a trace of melanine formation because, in normal cases, the melanine acts as a buffer against the oxygen of the air. If this oxygen, which penetrates into the sample during the preparation of the mash, were not eliminated by the formation of melanine, measurements would not be possible at all.

Tubers that show rust spots must be excluded from the very beginning. They cannot be measured. Their potential deviates toward the positive side and becomes constant in a range that does not correspond to their true cropping value. The same suspicion of an abnormal constant value should be applied to tubers infected with bacteria or fungi. Small visible infections can be cut out of the tuber. The formation of potential in the remaining part of the tuber is not influenced by this procedure. The greatest precautions must be observed in cases in which the infection is difficult to determine, as in the case of black ring. We know but little about these matters, but I believe that a number of failures must be debited to them, since we have as yet no other explanation.

Abnormal incidents in the measurements of tubers may appear for all varieties in autumn, that is, before winter dormancy. The curves of the measurements at first fall normally, then rise irregularly, and eventually fall again. In such measurements it is not possible to obtain constant values. This phenomenon is one of the most exceptional processes during the actual winter rest of the tuber. It may be observed again with a few, but not with all, varieties in the spring, when the winter dormancy is terminated.

All these errors that manifest themselves in the rising of the curves and in the lack of constant values are very rare. If one compares these cases in relation to their number with normal measurements, they appear almost negligible. I would like to emphasize this fact particularly because, in enumerating the great number of possible errors, you might be led to believe that the method is uncertain. Quite the contrary; because, by recognizing all sources of error, we have been able to guarantee this method to an extent that it is useful and valuable if applied by skilled workers.

Now, this still leaves the question as to the best time at which the measurements should be made and what part the properties of the different varieties may play. After the harvest in autumn, the tuber material must first be permitted to settle down to a certain state of inertia. The measurements cannot be carried out when, in spring, vegetation has begun. In both cases the unfitness of the material becomes evident by the fact that no constant values can be obtained. It is not possible to give an exact date of the beginning and the end of the "season." These dates depend not only upon the properties of the different varieties but also upon the sources of the seed. The degree of degeneration has to be taken into account also, as we were able to ascertain, particularly with some of the early varieties in which the degenerated tubers can no longer be measured, even from early in spring.

As already mentioned above, it became evident in the course of our investigations that not all varieties can be judged on an equal basis. We know today that there are several groups of varieties to which belong those most generally cultivated. In the first group we find the early variety of Erstling and Juliniere, furthermore, the Odenwälder Blaue, and the late variety of Wohltmann Broad Leaf, varieties that are by no means closely related to each other. The first group shows during winter dormancy the border line of healthy tubers at a potential value of calomel electrode minus 195 millivolts. A second group seems to comprise the majority of all varieties. To it belong the well known varieties Industrie, Direktor Johannsen, Parnassia, etc. During the period of winter dormancy, their vitality border lies at about Ec-165 mV. It is not yet certain whether there exists a third variety group, the border value of which lies at a different point. The variety Magnum Bonum, probably degenerated already at lesser values, seems to prove the existence of a third group. Although it is possible to say today that all potatoes, independent of the qualities of the varieties, are healthy if they show values up to electrode – 150 millivolts and diseased if the values are below electrode – 200 millivolts, the determination of the border values of the different varieties is of extreme importance in practice. Within the groups known to us, different sources of different varieties may be compared with regard to their cropping value. It is our aim to arrive at a comparison of the cropping values independently from the properties of the different varieties and to make such comparison on a known basis.

Of great importance, even for comparative measurements of the sources of one special variety, is the length of the measuring time. I have already mentioned that there is such a thing as season. This season, however, comprises a longer period than actual winter dormancy would require. The same basis of an immutable vitality border, however, exists in all varieties only at the time of complete inertia of the tuber, namely, during the actual

winter rest. Comparative measurements have shown that in autumn, when the possibility of obtaining constant values is given, the border values of the different varieties are at first somewhat higher. They then decline monthly by 10 mV. until winter rest begins, and remain, according to the species, at the same level for from 3 to 5 months, to drop again 10 mV. monthly in the spring of the year. Just as duration and temporal position of winter rest, so also the variability of the border values and their extent depend upon the properties of the different varieties.

You can see by these special questions that I have just discussed that Wartenberg and Hey, in their method of determining cropping values, have not quite found the solution of all problems. This is quite natural, since, in working on biological problems, out of every solved problem, new unsolved ones are arising. But, inasmuch as we are practical men, we are concerned to know how far the present methods are of practical value.

Determinations of the cropping value of seed that have been carried out in the Biologic Reich Institute with best results for practical use, have proved that this method is quite satisfactory even today. We divide the values into 3 groups: the vital group, the group of critical cases, and the group of degeneration. This division is somewhat more liberally defined than it is possible to determine the cropping values of seeds by actual measurement. This is of practical importance insofar as the effect of the cropping value not only depends on the state of health of the tubers, but also on the ecological conditions of the localities where they will be cultivated next year. The vitality group comprises potatoes, the potential values of which indicate perfect health. This may be ascertained, even today, with absolute certainty. With similar accuracy can be recognized seed material belonging to the degeneration group. The critical cases are critical in a double sense. First, their values lie near the border of vitality, so that it is rather difficult to judge whether the tuber is healthy or not, particularly if the border value of the variety in question has not yet been exactly determined. One must be careful in operating with cases approaching the border value, even if the latter be known; for the border value is but an expression of the transitionary zone. So, there will always be some uncertainty with regard to any judgment about values lying within the critical zone. This uncertainty increases because the ecological conditions of the cultivation ground may either augment the degeneration of the material, or inversely promote its recovery under favorable conditions.

What number of individual determinations is necessary in order to make the result of a test of one certain source conform to actual facts?

With a total number of 100 to 120 tubers, all possibilities and their frequency may be determined. This comparatively large number, therefore,

assures also the greatest certainty. A number of 50 or 60 tubers will show the types that may be expected, as well as a satisfactory indication of their frequency. The examination of only 20 to 30 tubers, however, enables one to say merely whether a certain seed material will produce degenerated plants. It may happen that the prognosis based on such a small number of tubers is faulty. But the probability of an error in experimenting with 30 tubers only is so small that even with this number, the question may be satisfactorily settled by the method of Wartenberg and Hey whether there is a considerable number of degenerated tubers among the material used in the determinations.

A FUSARIUM DISEASE OF CEREUS SCHOTTII

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INTRODUCTION

Besides the many species of Fusarium that have been reported as causing diseases of economic plants, there has been some work done on the relation of *Fusarium* spp. to diseases of certain ornamentals. *F. conglutinans callistephi* causes wilt in the China aster (3); *F.* sp., a stem rot of aster (1); *F. dianthi* Prill. and Delac., a stem rot of carnation (2); *F.* sp., wilt of carnation (7); and Fusarium rot, caused by *F. oxysporum* var. *gladioli*, is an important disease of gladiolus corms (4). The cacti, extensively used as ornamentals, are subject to various diseases. This paper deals with one of these diseases and the causal organism, which has been found to be a Fusarium.

HOST

Cereus schottii, the host plant of the Fusarium studied, grows in Sonora (Mexico), southern California, and southern Arizona. The diseased plant from which the fungus was first isolated was brought from Sonora, near Magdalena. An identical Fusarium was later obtained from a plant growing in the cactus garden of the University of Arizona. Since the latter had been placed in the garden nearly a year before it showed signs of disease, it seems doubtful that it was infected when placed in the garden. Therefore, it appears safe to say that the disease occurs in the northern and southern limits of the area in which the host is found. No data are available as to whether or not the disease occurs in California.

SYMPTOMS

The diseased plant from which the Fusarium was isolated showed a necrosis of the rot type. A large, slightly sunken lesion covered the entire lower half of the stem. This lesion was fuscous to fuscous black in color with a narrow area of light green yellow, 3 mm. in width, around the margin.

The interior of the infected part of the plant was colored fuscous to fuscous black. All the tissues were involved, so it was impossible to judge what part of the plant was first attacked or the manner in which the parasite entered and made its way through the plant.

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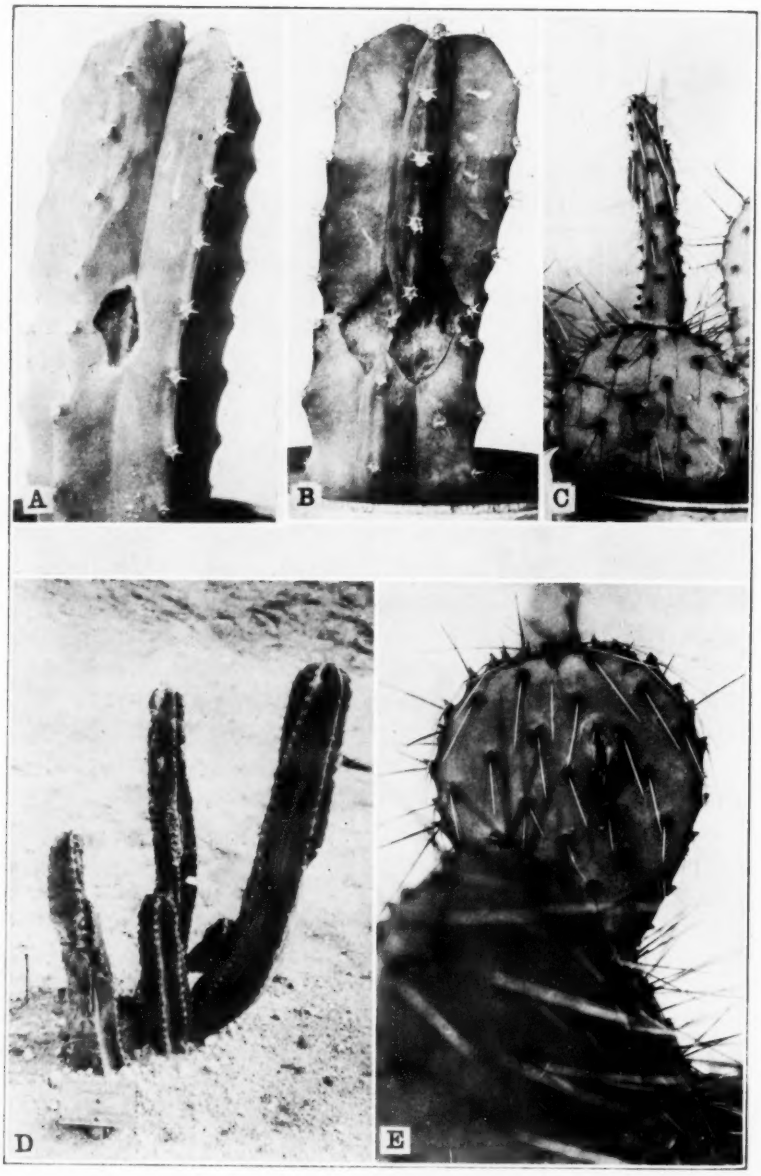

FIG. 1. A. Plant of *Cereus schottii* 10 days after inoculation, showing fuscous lesion with light-green margin. B. Same plant 24 days after inoculation. Corking off of lesion has begun. D. Plant of *C. schottii* from which the Fusarium was isolated. C. and E. Prickly pear (Engelmannii group), showing swelling and beginning of discolorations as result of inoculation. C. Profile of the swelling. E. Surface view.

CAUSE

a. Isolation

In the fall of 1931 a Fusarium was isolated from a plant of *Cereus schottii* (Fig. 1, D), in the following manner. The plant was broken open and bits of the diseased tissue cut out with a sterile scalpel and placed on slants of potato agar containing 2 per cent dextrose. In most cases a pure culture of the Fusarium was obtained. Since plants of *C. schottii* were not available at this time, the pathogenicity of the fungus was then determined by inoculating various species of Opuntia, chiefly of the *Engelmannii* group.

Monosporidial cultures were then obtained in the following way. A thin suspension of spores was made and kept at a temperature just above the solidification point. The agar containing spores was drawn up into a sterile capillary tube by means of a rubber syringe bulb. The contents of the tube, after being allowed to cool, were examined under the microscope and single spores separated from any others were marked. The pieces of glass tubing containing single spores were then cut out, dipped in 1–1000 mercuric chloride solution, and placed on agar slants. The cultures thus obtained were used for all future work.

b. Reactions

1. Potato—2 per cent Dextrose Agar, pH 7.2–28° C. Mycelium was well developed. Aerial hyphae present, 3 to 5 mm. in height, of a loose, cottony appearance, becoming slightly more compact and appressed with age; pure white in young cultures, becoming a pale pinkish buff with age. Discoloration of the medium began in 7 to 10 days. In a 21-day culture, the medium was bordeaux to dahlia carmine. Hyphae within the medium were not colored. Blue black sclerotia were found in 3-day and older cultures.

Lowering the pH to 5.6 produced the same results as those given above, except that growth was somewhat retarded. Potato- 5 per cent and 10 per cent dextrose agar, pH 7.2, were used with same results as given above, except that growth was slightly increased.

2. Autoclaved White Corn Meal, 28° C. (*Fig. 2, I*). The growth was much like that on potato-dextrose agar but slightly more compact in young cultures and never over 3 mm. in height. Medium was colored magenta to dahlia carmine, beginning on the 5th day and reaching bottom of plate in 12 days. The rate of growth on white corn meal was as follows:

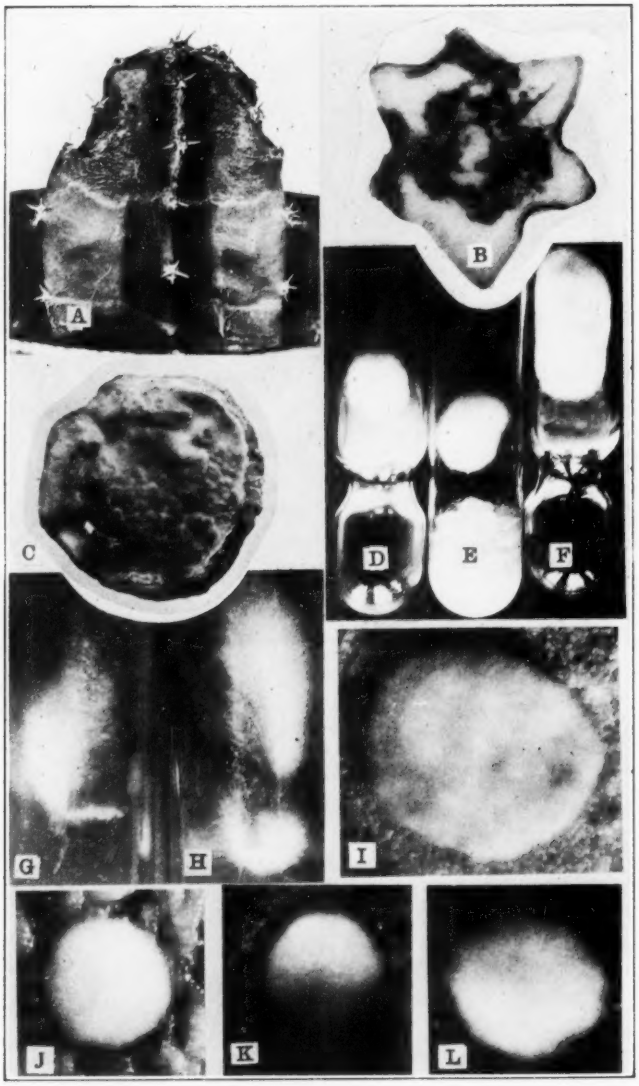

FIG. 2. A. Apex of plant of *Cereus schottii* three weeks after inoculation, showing discoloration. Cork tissue was not developed. B. Cross section of plant in A with discoloration of vascular system and tissues adjacent to it. C–L. 6-day cultures. C. On slice of living sweet-potato root. ×.5. D. On steamed potato plug. ×.79. E. On steamed carrot plug. ×.79. F. On steamed watermelon plug. ×.79. G. On steamed cotton stem. ×.88. H. On steamed watermelon stem. ×.88. I. On autoclaved white corn meal, 27° C. ×1.13. J. On autoclaved rice, 27° C. ×1.13. On oatmeal agar, 27° C. ×1.12. L. On yellow corn meal, 27° C. ×1.12.

Age of culture	Diam. of culture
24 hrs.	No perceptible growth
48 "	1 mm.
3 days	10 "
4 "	13 "
8 "	19 "
12 "	32 "
16 "	45 "

3. Autoclaved Yellow Corn Meal, 28° C. (*Fig. 2, L*). Same as white corn meal. Rate of growth slightly differed from above from day to day, but the growth over a period of 16 days was the same as given for white corn meal.

4. Oatmeal Agar, 27° C. (*Fig. 2, K*). Same as above.

5. Steamed Rice, 28° C. (*Fig. 2, J*). Growth more compact, less fluffy than on potato-dextrose agar. Pale hortense violet color of medium appeared around the edge of the colony after 5 days. In 14 days color reached bottom of Petri dish. Color of medium in 14-day culture, pale hortense violet to mulberry purple. In 60-day culture, the medium was a dull violet black. Hyphae within the medium, not discolored.

Rate of growth, slightly more rapid than that given for white corn meal. The growth after 16 days measured 5 cm. in diameter.

6. Nutrient Agar, pH 7.2, 28° C. Aerial mycelium well developed, 3–5 mm. in height, of a loose cottony appearance. Mycelium was pure white in young cultures. With age, growth appeared more compact and pale pinkish buff. No discoloration of the medium. Growth was slow on this medium as compared with that on potato-dextrose agar, yellow corn meal and white corn meal.

On nutrient agar, pH 5.6, growth was very slow and aerial mycelium not so well developed as at pH 7.2.

7. Steamed Potato Plugs, 28° C. (*Fig. 2, D*). Aerial mycelium much more compact, less fluffy than on any of the media described above. Growth was raised only slightly above the surface of the medium and was rather slow as compared with that on potato-dextrose agar. On the fifth day, medium became discolored a deep livid purple. By the 16th day the entire potato plug was colored deep livid purple, later turning dark violet gray.

8. Autoclaved Watermelon Stem, 28° C. (*Fig. 2, H*). On second day the growth appeared as a small tuft of erect, delicate, white mycelium from the point of inoculation. In 10 days erect, delicate hyphae spread over the surface of the stem and sides of the test tube; mycelium more compact than on 2-day culture but never so much so as on potato-dextrose agar, corn meal, or rice. Growth more rapid than on any other medium, except watermelon stem. Mycelium pale pinkish buff with age.

9. Autoclaved Cotton Stem, 28° C. (*Fig. 2, G*). Same as above.

10. Czapek Agar, 28° C. Growth slow as compared with potato-dextrose agar; appearance of culture as given for potato-dextrose agar; discoloration of medium beginning on seventh day, light neutral red.

11. Two Per cent Glucose Agar, 28° C. Appearance of culture much like that on potato-dextrose agar but slightly more compact; height of aerial mycelium not exceeding 3 mm. In 10 days medium was discolored tawny, later turning to ochraceous tawny and orange cinnamon. Aerial mycelium, pure white in young cultures turning pale ochraceous buff with age.

12. Slices of Living Sweet-Potato Root, 27° C. (*Fig. 2, C*). In 6 days most of surface was covered with white mycelium, not uniform in height, but ranging from 1 mm. to 5 mm. Mycelium turned light dove gray with age. Rot of tuber produced, fuscous in 21-day culture.

13. Living Whole Potato Tuber, 27° C. No growth.

14. Living Whole Sweet-Potato Root, 27° C. White mycelium from point of inoculation. After 5 days aerial mycelium became a light dove gray. Fuscous rot of tuber produced.

c. Microscopic Appearance of Hyphae

Hyphae from the various cultures described were examined in stained sections of a culture on potato-dextrose agar and in stained sections of the diseased stem of *Cereus schottii*. Sections were stained in Heidenhain's iron-alum haematoxylin and with light green in clove oil and saffranin. Hyphae were simply branched. The diameters of 25 vegetative cells from a culture on 2 per cent potato-dextrose agar averaged 2.43 μ. The average length of 25 such cells was 25.8 μ.

Tests with Sudan III and osmic acid on hyphae taken from a culture on autoclaved white corn meal showed numerous oil globules in most cells. A negative test for glycogen was given with potassium iodide. Cells that had most of their protoplasmic contents and had become extensively vacuolate were common in all cultures; only a thin layer of protoplasm remained around the cell wall in some cases. Swollen hyphal cells were numerous in all cultures. Some of these cells closely resembled chlamydospores, but they showed only a single wall (Fig. 3, A).

d. Reproduction

Microconidia and macroconidia (Fig. 3, E–G) were found in cultures. Microconidia, both ovoid and sickle-shape, were numerous in young cultures on all the media used. In the first cultures examined, nonseptate spores were produced almost exclusively on all media. When septate spores were transferred from sporodochia, which occurred occasionally, cultures pro-

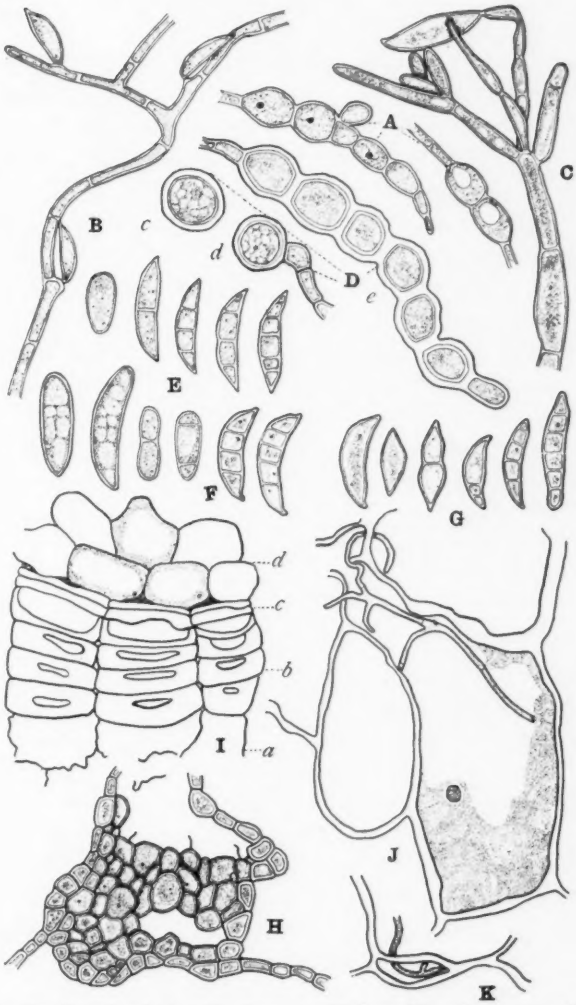

FIG. 3. A. Swollen hyphal cells from a 52-day-old culture on white corn meal. ×1060.4. B. Part of a conidiophore from a 40-day-old culture on 2 per cent potato-dextrose agar, pH 7.0. ×1060.4. C. Conidiophore from a 104-day-old culture on white corn meal. ×1060.4. D. Group of chlamydospores: *c* and *d*, from a 42-day-old culture on 2 per cent potato-dextrose agar, pH 7.0. ×1060.4; *e*, chlamydospores in a chain from a 42-day-old culture on steamed cotton stem. ×1060.4. E. Group of spores from a 51-day culture on white corn meal. ×1060.4. F. Group of spores from a 104-day culture on white corn meal. ×1060.4. G. Group of spores from a 90-day culture on 2 per cent potato-dextrose agar, pH 7.0. ×1060.4. H. Section of a sclerotium. ×1060.4. I. Host tissue: dead parenchyma cells (*a*), cork cells (*b*), cork cambium (*c*), and normal cells (*d*). ×154. J. Intracellular hypha. ×330. K. Hypha in intercellular space. ×330.

ducing septate spores were obtained. Microconidia were not borne in sporodochia nor pionnotes.

Macroconidia were produced in sporodochia that were confluent in nature. Conidial masses were pale vinaceous pink. Macroconidia were gradually attenuate at both ends, usually uniformly curved throughout; apedicellate, or with the foot poorly developed. Macroconidia were 3–5 septate.

Nonseptate chlamydospores, both terminal (Fig. 3, D, *d*) and intercalary (Fig. 3, D, *e*), were found in cultures on potato-dextrose agar, autoclaved potato plugs, corn meal, rice, and autoclaved stems. Although chlamydospores were found without difficulty, they were never abundant. The average diameter of 10 such spores, measured through the widest part of the spore, was 15.2 μ. Cells differing from true chlamydospores, in that they had only a single wall, were very numerous in all cultures (Fig. 3, A).

Blue black sclerotia were abundant (Fig. 3, H). In cultures 30-days old on potato- 2 per cent-dextrose agar numerous sclerotia were found imbedded in the medium. In cultures 70 days old, on autoclaved rice and corn meal, sclerotia were so numerous that the remaining medium appeared blue black. Sclerotia varied in size up to 1½ mm. in diameter.

e. Identification

By comparison with the descriptions (6, 8) of the section *Elegans* of the genus Fusarium, it is evident that the fungus belongs in that group. The fungus described in this paper agrees with these descriptions in the presence of both microconidia and macroconidia; in the occurrence and size of terminal and intercalary chlamydospores and blue black sclerotia; in the degree of development and color of aerial mycelium and in the discoloration of media. However, 3-septate conidia are given in the descriptions as typical for this group while 4- and 5-septate conidia were found to predominate in 2 groups of 100 spores measured in this study.

Within the section *Elegans*, the fungus falls close to *Fusarium oxysporum*, but there are certain differences that appear to be of sufficient importance to set it apart as a variety of *F. oxysporum*. The fungus agrees with *F. oxysporum* in the following characteristics. Conidiophores are typically much branched (Fig. 3, B and C). Sickle-shape microconidia are gradually attenuated toward the apex, nearly cylindrical and broadest in the middle half of the length. Macroconidia are borne in sporodochia. Conidial masses are pinkish buff. Aerial mycelium is white and well developed, of medium height, 3–5 mm., and discoloration of the substrate varies through shades of red and purple. Both terminal and intercalary chlamydospores are present, and also blue black sclerotia.

Characteristics that do not agree with Wollenweber's (8) and Sherbakoff's (5) descriptions of *Fusarium oxysporum* are as follows. The nonsep-

TABLE 1.—*Spore measurements*[a] *of organism obtained from diseased plants of Cereus schottii*

Medium	0-Septate	1-Septate	2-Septate	3-Septate	4-Septate	5-Septate
White corn meal 10-day	93 per cent 5.2–14.5 × 2.5–4 μ Av., 9.7 × 2.3 μ	6 per cent 15.5–16.5 × 3–4 μ Av., 15.3 × 2.3 μ	1 per cent 15.5–16.5 × 3–4 μ Av., 15.5 × 3.5 μ			
White corn meal 43-day	14 per cent 8–12 × 2–3 μ Av., 9.8 × 2.8 μ	18 per cent 8.5–9.5 × 2.5–3.5 μ Av., 8.8 × 2.7 μ	60 per cent 8–11 × 2.5–3 μ Av., 9.6 × 26 μ	8 per cent 9–12 × 2.8–3 μ Av., 10 × 2.5 μ		
2 per cent potato-dextrose agar pH 7 20-day	83 per cent 4.2–11.5 × 2–3 μ Av., 9.9 × 2.7 μ	4 per cent 9–14.2 × 2.5–4 μ Av., 11.5 × 3.8 μ	13 per cent 8–14.8 × 3–4 μ Av., 12.2 × 3.5 μ			
2 per cent potato-dextrose agar pH 7.2 63-day	4 per cent 9–12.5 × 2.8–3.9 μ Av., 11.8 × 3 μ	10 per cent 9–15.5 × 2.8–4 μ Av., 14.8 × 3.6 μ	4 per cent 9–16.8 × 3.2–4 μ Av., 15.2 × 3.6 μ	21 per cent 11.5–16 × 3–4 μ Av., 15.5 × 3.5 μ	9 per cent 14.2–17.2 × 3.2–4 μ Av., 15.2 × 3.5 μ	52 per cent 17.2–21.4 × 4–4.2 μ Av., 19.9 × 4.15 μ
Autoclaved cotton stem 32-day	5 per cent 10.5–13.5 × 2.5–3 μ Av., 12 × 2.7 μ	16 per cent 9–16 × 3.1–3.5 μ Av., 11.9 × 3.1 μ	7 per cent 10.5–16 × 2.8–3 μ Av., 14 × 2.9 μ	72 per cent 11.5–16.5 × 2.5–3.5 μ Av., 14.5 × 3 μ		
					(50 spores)	
Autoclaved watermelon stem 51-day	5 per cent 11–12.9 × 2.8–3.5 μ Av., 12.5 × 3.2 μ	6 per cent 9–16 × 2.8–4 μ Av., 15.3 × 3.9 μ	4 per cent 9–16.2 × 3.4–4 μ Av., 14.2 × 3.4 μ	15 per cent 11–16 × 3–4 μ Av., 15.4 × 3.9 μ	8 per cent 14.2–15 × 3–4 μ Av., 14.4 × 3.9 μ	62 per cent 16–17.2 × 4–4.2 μ Av., 16.3 × 4 μ

[a] Measurements are for 100 spores, except as indicated.

tate microconidia measured, taken from a culture on 2 per cent potato-dextrose agar, pH 7.0, averaged $9.9 \times 2.7\ \mu$ ($4.2–11.5 \times 2–3\ \mu$) as compared to $8 \times 2.9\ \mu$ ($4.5–12 \times 2–4\ \mu$). One-septate spores averaged $11.5 \times 3.8\ \mu$ ($9–14.2 \times 2.5–4\ \mu$) compared to $16.5 \times 3.4\ \mu$ ($11–23 \times 2.5–4.1\ \mu$). Two-septate spores averaged $12.2 \times 3.5\ \mu$ ($8–14.8 \times 3–4\ \mu$) compared to $18.5 \times 3.9\ \mu$ ($17–26 \times 3–4.2\ \mu$). Three-septate spores averaged $12.2 \times 3.5\ \mu$ ($8–14 \times 3–4\ \mu$) compared to $27.5 \times 4\ \mu$ ($15–39 \times 3–4.7\ \mu$).

Four-septate conidia occur only rarely, according to the descriptions of *Fusarium oxysporum* previously mentioned, never making up more than 3 per cent of the number of spores measured, while 8 and 9 per cent measured in 2 groups of 100 each in this study were 4-septate spores averaging $14.4 \times 3.9\ \mu$ ($14.2–15 \times 3–4\ \mu$) and $15.2 \times 3.5\ \mu$ ($14.2–17.2 \times 3.2–4\ \mu$), respectively, compared to $36.7 \times 4.43\ \mu$ ($34.5–60 \times 4.3–4.5\ \mu$). In the descriptions, 5-septate conidia make up 1 per cent of the spores measured, while in the 2 groups of 100 spores here studied the 5-septate spores predominated, the average size being $16.3 \times 4\ \mu$ ($16–17.2 \times 4–4.2\ \mu$) and $19.9 \times 4.15\ \mu$ ($17.2–21 \times 4–4.2\ \mu$) as compared to $35 \times 4.4\ \mu$ (no minimum and maximum given).

The spores of the fungus studied were either apedicellate or had the foot typically less well-developed than is characteristic of *Fusarium oxysporum*. There also is a marked difference in the parasitism of the 2 organisms. The Fusarium studied does not infect the Irish potato.

Therefore, because of the minor differences between *Fusarium oxysporum* and the organism described in this paper, the differences in spore size, the poor development of the foot, and the difference in parasitism, it would seem that the organism might be classified as a variety of *F. oxysporum*.

f. Effect Upon the Host

Inoculation of plants of *Cereus schottii* and prickly pear were made by cutting a small slit in the sterilized surface of the stem with a sterile scalpel and inserting a bit of monosporidial culture. Within 10 days a pronounced swelling appeared around the point of inoculation (Fig. 1, C and E). The raised area was discolored fuscous with a light green yellow margin. In 6 days the lesion had become sunken and had doubled in size. (Fig. 1, A, B). After 3 weeks, some of the plants had become fuscous or fuscous black almost throughout, while others had checked the progress of the organism by developing several rows of cork cells between the lesion and the normal tissue (Figs. 2, A, and 3, I). These thick-walled cork cells originated in the parenchyma.

Upon examination of plants that did not develop protective tissue the vascular system and parenchyma adjacent to it were found to be discolored the entire length of the stem. Stained sections of tissue at some distance

from the point of inoculation showed no hyphae present. These observations led to the conclusion that the fungus was excreting a substance or substances toxic to the living plant cells and that the toxic excretion was carried through the plant in the vascular system.

To further investigate this condition, a culture of the Fusarium was grown in nutrient broth containing 2 per cent peptone (6). After 8 days, the broth was filtered so that all spores of the fungus were excluded and the filtrate examined under the microscope to be certain of this fact. A cut end of a stem of *Cereus schottii* was then immersed about 1½ inches in the filtrate and allowed to remain there for 8 days. The stem was then cut longitudinally and examined. The first ¼ inch of stem above the cut surface was discolored fuscous throughout. Above this region only the vascular bundles were discolored a light brown. This discoloration of vascular tissues extended to the apex of the plant.

A cross section of the stem in the region of inoculation showed discoloration throughout. Sections of tissue in this region stained with Heidenhain's iron-alum haematoxylin showed the presence of both intercellular (Fig. 3, J) and intracellular (Fig. 3, K) hyphae. In the older part of the lesion, cells had entirely lost their protoplasmic contents and cell walls were badly broken down. Some sections showed only hyphae of the fungus and fragments of cell walls.

SUMMARY

A Fusarium was isolated from a plant of *Cereus schottii* which showed necrosis of the rot type. Inoculations proved the fungus to be pathogenic. Monosporidial cultures were then made.

The Fusarium was found to belong to the section *Elegans* and to fall close to *F. oxysporum* within that group. It differs from *F. oxysporum* in having smaller macroconidia, a higher percentage of 4 and 5-septate spores, in having spores with the foot less well-developed than is characteristic of *F. oxysporum,* and in its pathogenicity. For these reasons the fungus is tentatively classified as a variety of *F. oxysporum.*

When plants of *Cereus schottii* were inoculated with the Fusarium, symptoms were produced that were identical with those shown by the naturally diseased plants, and the fungus was reisolated.

Inoculated plants of *Cereus schottii* showed discoloration of the vascular system and adjacent cells. Discoloration of tissues extended beyond the cells containing hyphae of the fungus as shown by stained sections. This fact led to the conclusion that a substance (or substances) toxic to the cells of the host was being excreted by the fungus. The Fusarium was grown for 8 days in nutrient broth. The broth was then filtered and the cut end of a stem of *Cereus schottii* immersed in the filtrate. The results of this

experiment substantiate the belief that a toxic substance was excreted that affected the living cells of the host.

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EFFECT OF LIGHT ON THE DEVELOPMENT OF THE UREDIAL STAGE OF PUCCINIA GLUMARUM[1]

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INTRODUCTION

Considerable difficulty has been experienced in propagating stripe rust, *Puccinia glumarum* (Schmidt) Eriks. and Henn., in the greenhouse at Moscow, Idaho. In the earlier work of Hungerford and Owens (3), at Corvallis, Oreg., very little if any difficulty was encountered in propagating this rust under greenhouse conditions. Although the reason for this difference is not definitely known, it is thought to have been due to the difference in climatic conditions. It was, therefore, deemed fundamental and necessary to the undertaking of any intensive study of this fungus to determine the conditions necessary for its optimal development in the greenhouse. An investigation of this type, where light and temperature could be controlled, was necessary also before cultures of stripe rust could be satisfactorily carried through the hot, dry summer months. The object of this work, therefore, was (1) to determine the effect of photoperiodism on *P. glumarum;* (2) to determine the optimum light intensity for the best development of the fungus; (3) to ascertain whether successful infection could be secured by subjecting the plants to light immediately after inoculation without first placing them in the shade for 48 hours; and (4) to determine whether sporulation could be secured from plants inoculated with urediospores that had been developed under a low light intensity.

Wilhelm (9), in a study on the mode of specialization of the urediospores of *Puccinia glumarum*, found that a reduction in light intensity prevented full expression of the type of infection but did not affect the relative susceptibility of the different varieties. Stakman and Levine (7) and Peltier (6), working with *P. graminis* (Pers.), and Fromme (1), working with *P. coronifera* (Kleb.), secured corresponding results, viz., these cereal rusts

[1] Cooperative investigations between the Idaho Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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developed more luxuriantly in fairly high light intensities than they did in lower ones. Melander (4), working with *P. graminis tritici*, form 15, found that extremely high light intensities, as well as low ones, caused a reduction in the ability of the urediospores to withstand low temperatures.

EXPERIMENTAL METHODS AND RESULTS

The method of inoculating the plants was, with certain modifications, the same as that employed by Stakman and Piemeisel (8, p. 432) in their work with *Puccinia graminis*. A spore suspension was first made by placing a drop of water on an ordinary glass slide and then, with a scalpel, scraping the spores from an infected plant into the drop of water. A small flattened needle was used to apply the spores on the primary leaf, only. Immediately following inoculation the plants were covered with a bell jar for 48 hours. In this method it was found unnecessary to draw the leaf between the thumb and forefinger before inoculation to remove the waxy coating, or to atomize the leaves after the spores had been applied. Sufficient spore material was used for each inoculation to insure good infection. The plants were kept trimmed down to the inoculated leaf throughout the investigation. The tests were performed in a temperature-control room, completely devoid of daylight. Stock cultures were maintained in the greenhouse by means of artificial light. Pannier, C. I.[4] No. 1330, a variety of barley known to be completely susceptible to *P. glumarum*, served as the host plant for the study herein reported. Single-spore cultures were not employed because the work was primarily undertaken for the purpose of studying the development of the fungus and not its specialization. All inoculations, however, were made from a culture of *P. glumarum* collected at Moscow, Idaho, in the fall of 1932.

The experiments were carried on in a temperature-controlled room 6 ft. x 9 ft. x 8 ft. Three ordinary Mazda 200-watt and 2 100-watt bulbs, with a voltage of 115, provided the light for the room. The lights were placed 30 inches from the plants, admitting illumination only from above. Ordinary Westinghouse daylight bulbs were used in place of the above, with no difference in results.

PHOTOPERIODISM

In the study of photoperiodism, 35 6-inch pots containing 5 plants each, or a total of 175, were inoculated as described above. At the termination of the infection period (48 hours after inoculation) the pots were grouped, so that there were 7 sets of 5 pots each. The inoculated plants were exposed, respectively, to 6, 8, 10, 12, 15, and 24 hours of light per day under identical temperature conditions. In other words a set of 5 pots was ex-

[4] C. I. denotes accession number of the Division of Cereal Crops and Diseases.

to each of the above light periods. One set was placed in ordinary light as a control. The plants were subjected to the above treatment for 32 days after inoculation.

In the various light exposures, supposedly comparable to different day lengths, little difference was observed up to a certain exposure (Figs. 1, 2 and 3 A). There was a direct relationship between the time in which sporulation appeared and the length of exposure. This relationship also held true in the subsequent development of the rust. With 6 hours of light per day, the rust was considerably slower, both in sporulation and development, than that of the 8-hour day and so on, up to the 12-hour day. More than 12 hours of light per day gave a distinct change in rust type, as shown in table 1 and figure 3 A.

TABLE 1.—*The effect of different day lengths on the length of incubation period and type of infection produced by Puccinia glumarum on Pannier barley, C. I. No. 1330*

Temperature Min.	Max.	Mean	Day length	Period of incubation	Plants inoculated	Plants infected	Type of infection[a]
° *F.*			*hrs.*	*days*			
45	50	47.5	6	20	25	21	4
45	50	47.5	8	16	25	20	4
45	50	47.5	10	13	25	24	4
45	50	47.5	12	9–11	25	24	4
45	50	47.5	15	12	25	19	0
45	50	47.5	continuous	12	25	15	0
50	55	52.5	ordinary day	21	25	3	4

[a] 4 = completely susceptible; 3 = moderately susceptible; 2 = moderately resistant; 1 = very resistant; 0 = extremely resistant; I (or) − = immune.

It will be noticed from the above table that, up to a certain point, the day length does not affect the type of infection. It does, however, change the length of the incubation period considerably and the subsequent development of the rust (figs. 1, 2). A 6-hour day, in comparison with the 12-hour day, lengthens the period of incubation 9 days. As the day length increases, the period of incubation decreases up to a certain point. There is essentially no difference in the length of the incubation period when the day length is 12 hours or longer. It also will be noticed that a day length greater than 12 hours changes the type of infection from a type-4 to a type-0. In the case of the ordinary day it will be noted that the temperature was a little higher and the period of incubation longer than in the other part of the experiment. The higher temperature resulted from failure to

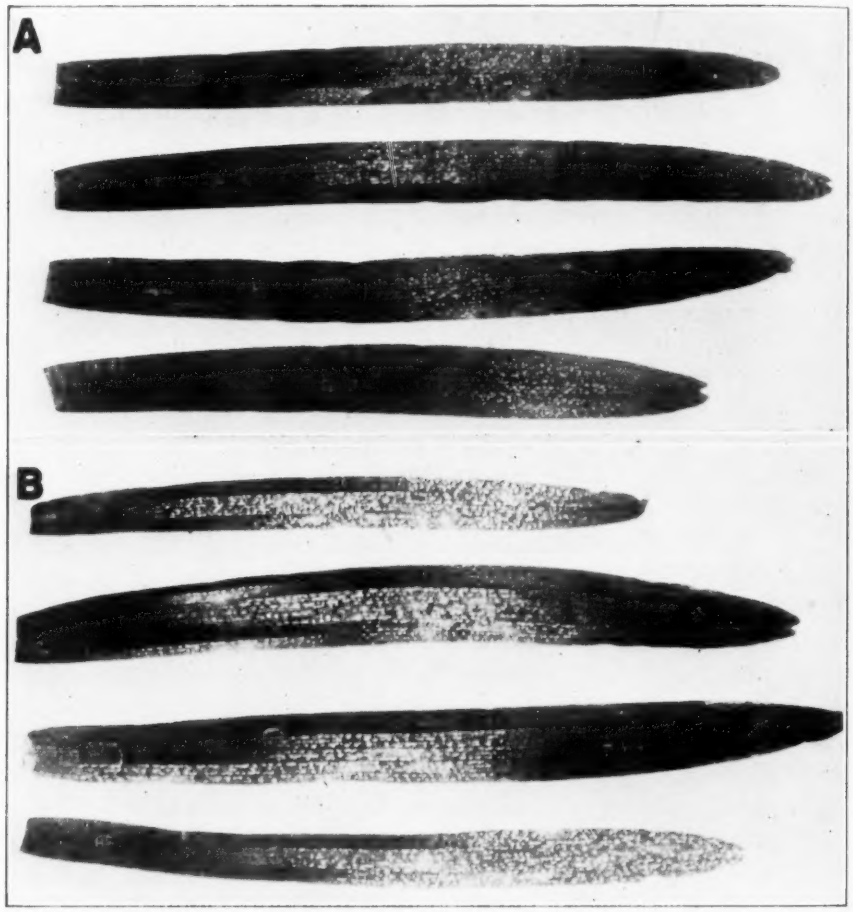

FIG. 1. Thirty-two-day-old cultures of *Puccinia glumarum* on leaves of barley plants exposed at 45–50° F. to light for six hours per day (A) and for eight hours per day (B).

control the temperature as accurately in the greenhouse as in the temperature-control room. The longer incubation period in case of the ordinary day was caused by the lowered light intensity of the cloudy days of the winter months while the work was in progress. The writer has often found it difficult to propagate *Puccinia glumarum* in the greenhouse during winters of little sunshine, while in winters of considerable sunshine it can be easily propagated. This fact substantiates the data given in table 1. The above experiment was repeated at a temperature range of 55 to 60° F. with essentially no difference in results. At temperatures of 68 to 70° it was found necessary to keep bell jars over the plants and the soil in the pots

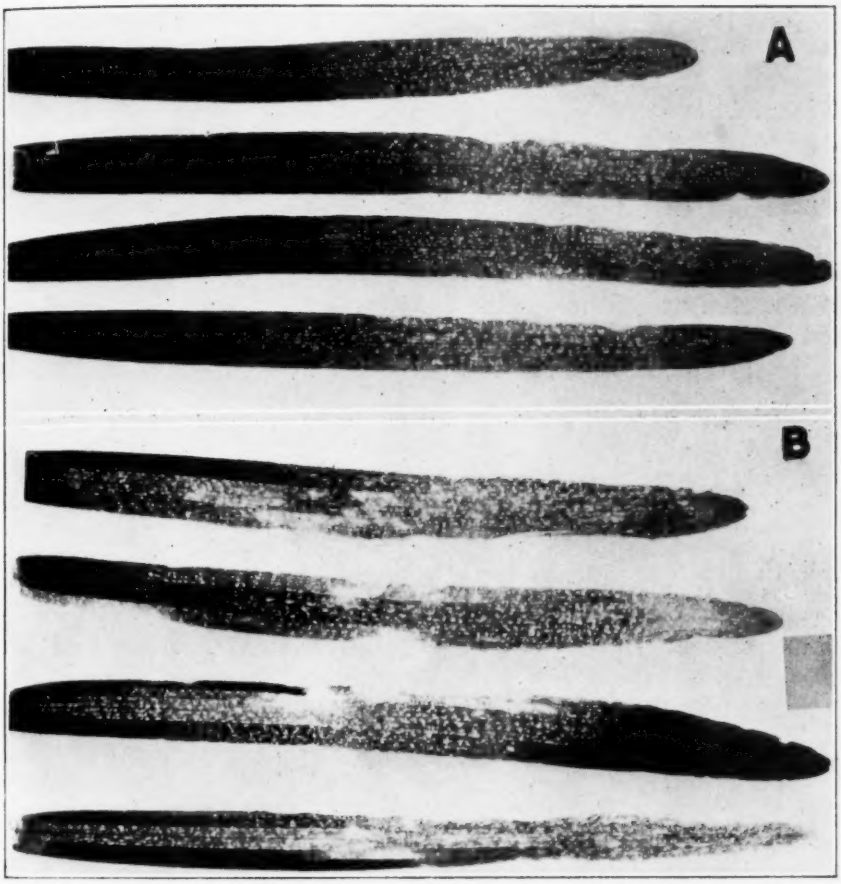

FIG. 2. Thirty-two-day-old cultures of *Puccinia glumarum* on leaves of a barley plant exposed at 45–50° F. to light for 10 hours per day (A) and twelve hours (B).

thoroughly wet to secure any sporulation at all. Under these conditions a type-4 infection was obtained. At temperatures above 70° very little infection developed and that little belong either to type 2 or 3. At a temperature of 80° no infection was secured. These results accord essentially with those obtained by Newton, Johnson, and Brown (5) in their studies on stripe rust in Canada.

No attempts have been made definitely to explain this phenomenon. Garner and Allard (2), however, have found in their study of other types of plants that a day length of over 12 hours has a tendency to change the nitrogen-carbohydrate ratio in the plant. In other words, there is a translocation of plant constituents. On this basis it was thought that possibly the same might hold true in the case of *Hordeum vulgare* (L.) and, hence,

the change in resistance of the plant. It is a well-known fact that the rust fungus is primarily a carbohydrate feeder; therefore, any treatment that would cause a change in the nitrogen-carbohydrate ratio in the host plant might create an apparent resistance to the parasite that would not otherwise occur. This explanation as a probable cause of the change in rust type cannot be considered conclusive. Further experimentation is necessary and a complete chemical analysis of the plants must be made before any definite conclusion can be drawn. Another possible explanation of this phenomenon is the fact that a long period of light may have a lethal effect on the rust mycelium in the tissue. This is easily conceivable because the leaf tissue of barley seedlings is very thin, allowing considerable light to pass through. One must bear in mind, however, that the effect of the light is lethal only to the extent of inhibiting sporulation. The rust mycelium continued to grow during the entire period of the experiment but produced no spores.

LIGHT INTENSITY STUDIES

In these studies the same general procedure was followed as that described in the foregoing discussion. Each of a set of 5 pots containing the inoculated plants was subjected to a different light intensity. One set of inoculated plants was exposed to 960 foot candles of light supplied by a 1000-watt lamp; the second set was exposed to 576 foot candles of light supplied by 3 200-watt lamps; the third set to 384 foot candles of light supplied by 2 200-watt lamps; the fourth to 192 foot candles supplied by a 200-watt lamp and the fifth set to 96 foot candles supplied by a 100-watt lamp. When more than one lamp was used to supply the necessary intensity, they were so slanted that the resulting light was concentrated into a circle. The plants were exposed to light 9 hours daily.

TABLE 2.—*The effect of light intensity on the subsequent development of Puccinia glumarum on Pannier barley*

Temperature			Light intensity		Incubation	Plants inoculated	Plants infected	Type of infection
Min.	Max.	Mean						
° *F.*			*Watts*	*Foot candles*	*Days*			
45	50	47.5	1000	960	11	25	22	4
45	50	47.5	600	576	12	25	25	4
45	50	47.5	400	384	13	25	20	4
45	50	47.5	200	192	13	25	20	4
45	50	47.5	100	96	16	25	18	4
50	55	52.5	ordinary day		20	25	24	4

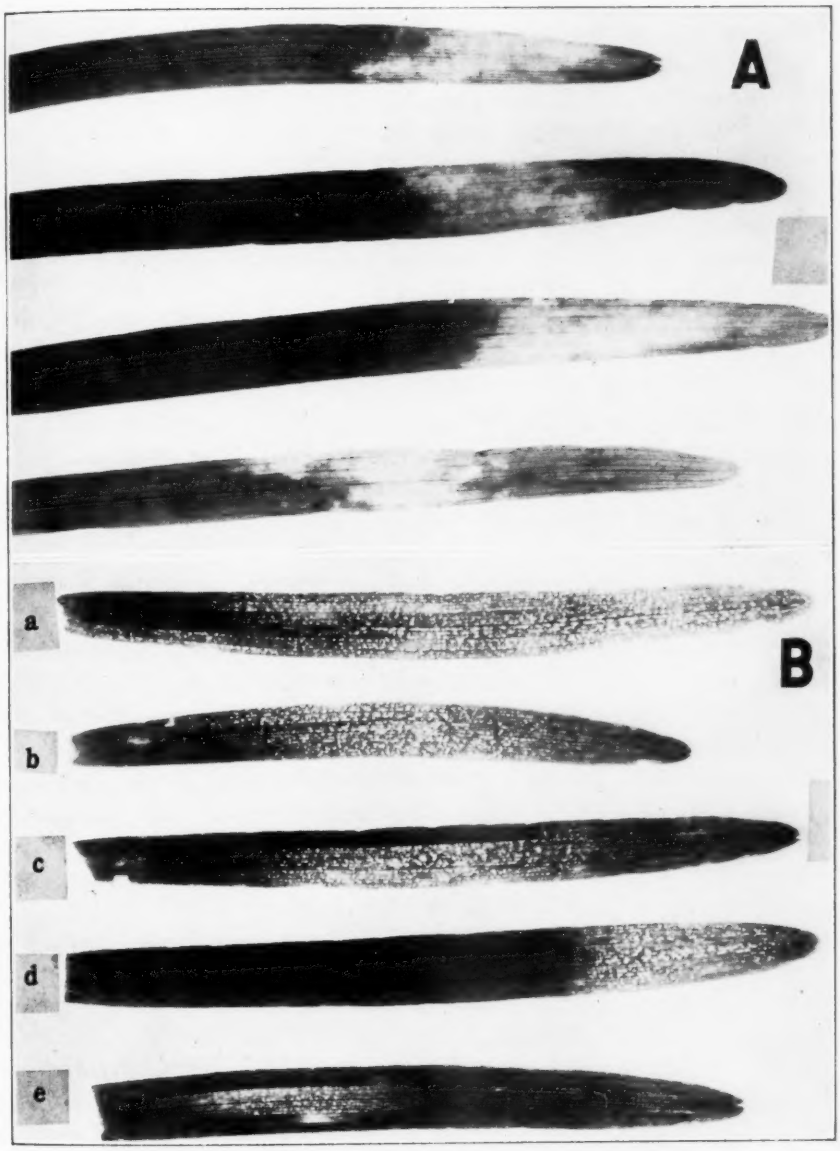

FIG. 3. A. Thirty-two-day-old cultures of *Puccinia glumarum* on leaves of barley plant exposed to continuous light at 45–50° F. B. Twenty-eight-day cultures of *Puccinia glumarum* on leaves of barley, showing the effect of light intensity on the subsequent development of the rust fungus: a, culture grown in light intensity of 960 foot candles; b, of 576; c, of 384; d, of 192; and e, of 96.

Light intensity has essentially the same effect on the development of urediospores of *Puccinia glumarum* as does the day length.

Table 2 shows in detail the results of the light-intensity studies.

Table 2 and figure 3, B show the effect of light intensity on the development of *Puccinia glumarum*. As in the case of the day length, the light intensity has its corresponding effect on the incubation period; that is, the higher the intensity, the shorter the incubation period. The light intensities employed in these investigations, however, did not change the rust type as did the longer day length. In general, these results agree with those of Stakman and Levine (7), Peltier (6), and Fromme (1).

In studying *Puccinia glumarum* in the greenhouse it has been the general practice to cover the newly inoculated plants with a bell jar and place them in a rather dark, shady place for 48 hours. This was done primarily to keep the humidity sufficiently high for germination and infection. It was thought that time and space could be conserved if the newly inoculated plants were exposed to the light immediately after inoculation. For this test 25 such plants were covered with bell jars and immediately placed in a compartment of medium light intensity. Other inoculated plants were placed in semi-darkness as controls. The bell jars were removed from all of the plants at the end of 48 hours.

Excellent infection was secured by subjecting the plants to light immediately after inoculation, a fact that is quite contrary to earlier teaching that plants inoculated with *Puccinia glumarum* should first be kept in a shaded place for 48 hours before subjecting them to the desired conditions. To obtain infection, however, it is necessary to cover newly inoculated plants with bell jars for 48 hours following their inoculation. Knowledge of this fact will enable one to conserve both time and space in one's study of this fungus. It will be especially helpful in varietal testing in the greenhouse, as well as in the study of physiologic forms.

Wilhelm (9), in his work on *Puccinia glumarum*, found that urediospores obtained from plants deprived of sufficient light were not viable and, therefore, were incapable of causing infection. In a similar study the writer inoculated 25 plants with urediospores that had been developed under a low light intensity (96 foot candles). These inoculated plants were placed in a compartment of higher light intensity (576 foot candles) and left for 15 days. Check plants were inoculated and placed in semi-darkness.

Contrary to the results of Wilhelm (9), good infection resulted when plants were inoculated with urediospores that had been developed under low light intensity (light produced by a single 100-watt lamp). No germination was secured in water cultures of urediospores of *Puccinia glumarum* that had been formed in such low light intensity. From previous experiences,

however, the percentage of germination of urediospores in culture is no positive criterion as to whether infection can or cannot be secured when plants are inoculated with urediospores that fail to germinate in culture.

In all of the above described tests, the temperature range was from 45° to 50° F. In no case was any special attempt made to regulate the humidity, although a record of the humidity was kept by means of a hygrothermograph.

DISCUSSION

It has been observed from time to time that the spring advent of *Puccinia glumarum* is very tardy. In checking over the weather records and the spring-advent dates of *P. glumarum*, it was found that in an unusually cloudy and rainy spring stripe rust appeared very late. The temperatures recorded for corresponding years of considerable cloudy weather did not vary sufficiently to explain the difference between advent dates of a cloudy spring and those of a sunny one. Therefore, in the light of the results herein presented, it would be safe to presume that in the case of considerable cloudy weather, the light intensity may be sufficiently decreased to delay the appearance of *P. glumarum*. This late initial appearance of stripe rust does not occur in springs characterized by a large percentage of sunny days.

SUMMARY

The day length, up to a certain number of hours, caused no change in the expression of the infection type of *Puccinia glumarum* at temperatures of 45° to 50° F. and 55° to 60° F. The shorter day increased the necessary incubation period.

The 6-hour day, compared with the 12-hour day, increased the incubation period 9 days. There was no further change in the period of incubation when the day length was increased beyond 12 hours.

When the day length exceeded 12 hours, there was a distinct change in the infection type from type-4 to type-0 (Fig. 2).

A high light intensity decreased the period of incubation and increased the subsequent development of the fungus.

Low light intensity did not permit the full expression of the rust; there was, however, no change in the infection type.

There was essentially no difference in the development of the fungus in the light intensities supplied by 400-watt and 600-watt bulbs.

Excellent infection was secured by subjecting plants to the lights immediately after inoculation rather than shading them for 48 hours before placing them under the lights. In all cases it was necessary to place a bell jar over the newly inoculated plants for the 48-hour period, to maintain the relative humidity necessary to spore germination.

Urediospores that were produced under a low light intensity failed to germinate in culture. When plants were inoculated with such spores, good infection resulted.

No attempt was made to control the relative humidity. It ranged between 58 and 92 per cent, as indicated by hygrothermograph records.

There was no difference in results in temperatures of 45° to 50° F. and 55° to 60° F. In temperatures of 68° to 70° F. the infection type was changed from a type-4 to a type-2 or 3. No infection was obtained at 80° F. or above.

No germination was secured in cultures of urediospores of *Puccinia glumarum* that had developed under low light intensity. Good infection, however, was obtained when plants were inoculated with spores from the same lot.

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A SURVEY OF POTATO SCAB AND FUSARIUM WILT IN WESTERN NEBRASKA[1]

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INTRODUCTION

The two most serious diseases of the potato in the commercial seed-producing sections of western Nebraska are potato scab (*Actinomyces scabies* (Thax.) Güssow) and Fusarium wilt and stem-end rot (*Fusarium eumartii* Carp. and *F. oxysporum* Schlect.). Satisfactory methods of controlling these diseases have not been developed for the reason that most of the infection comes from the soil.

As a preliminary step in the investigations of the effect of various soil factors upon the occurrence of these two diseases the author undertook a survey of fields in western Nebraska in the summer of 1928. While the chief aim of the survey was to obtain data that would aid in outlining future experimental work, some of the records were thought to be of general interest and are here presented.

SURVEY METHODS

In order to eliminate variation caused by infected seed tubers, 100 bushels of Bliss Triumph certified seed were selected from a single lot of seed potatoes and treated with hot formaldehyde (1 to 120) for 4 minutes at 124° F. One bushel of this treated seed was then sent to each of 100 growers of certified potatoes in western Nebraska. Each grower planted the bushel in one or more rows through the middle of a commercial field, otherwise planted to commercial certified seed treated with hot formaldehyde. The fields were selected as representing the widest range of environmental conditions existing in the potato-growing sections of the western 9 counties of the State. All but 4 fields were on dry-land in the high plains area, and these 4 exceptions were irrigated fields in the Scotts Bluff section.

Field Records. The following data were recorded for each field: soil type, the number of years the soil had been under cultivation, the crops grown for the preceding 5 years, and any such special treatments as manure, the preparation of the land, and number of cultivations and dates of planting and harvesting.

Disease Records. At harvest time each field was visited and a 100 pound random sample was taken from the rows used for the test. All

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potatoes, regardless of size, were examined individually for scab and stem-end rot or vascular discoloration. The disease grades were as follows: *Scab Grades; Healthy; Slight, i.e.,* not more than 1 spot $\frac{1}{4}$ inch in diameter or 5 spots $\frac{1}{8}$ inch in diameter; *Medium, i.e.,* more than *Slight* but less than $\frac{1}{4}$ of the surface of the tuber; *Severe, i.e.,* more than $\frac{1}{4}$ of the surface. Superficial scab occurring without definite corky lesions was also recorded. In this paper the term total scab refers to all of the above 4 types or degrees of severity.

Fusarium Grades:—Healthy; Slight, i.e., not more than $\frac{1}{4}$ inch deep; *Medium, i.e.,* $\frac{1}{4}$ inch to $\frac{1}{4}$ of the length of the tuber; *Severe, i.e.,* more than *Medium.* In addition to the above notes the type of infection also was recorded as vascular discoloration, stem-end rot, or a combination of these 2 types. Throughout this paper the term Fusarium wilt will be applied to tubers showing symptoms of the above types. All disease records were made on the basis of weight rather than number.

Weather Data. Temperature and rainfall records were obtained from the United States Weather Bureau records for 8 stations located within the survey area. In addition, rain-guages were used at five other points in this area.

Soil Data. In late July and early August each field was visited and 2 sets of soil samples were taken, one for the determination of soil type, organic matter, and hydrogen-ion concentration,[2] and the other for the determination of the numbers of bacteria and Actinomyces and other fungi. The latter samples were taken in the row and between the hills with a 2-inch sterile tube to a depth of 6 inches, the loose surface soil first being removed. Three composite samples of 5 cores each were obtained from each test row. After thorough mixing a portion of each sample was placed in a sterile pint jar and the jars were immediately shipped to Lincoln, where they were either plated at once or placed in a cold-storage room held at a temperature of 36° F. Most of the samples were plated within a few days but it was necessary to hold some of them for a longer period of time up to one month. As it turned out, this introduced a considerable error when individual fields were compared[3] but the average storage periods of the samples as grouped for the tables that follow are practically the same.

[2] The writer is indebted to J. C. Russel of the Agronomy Department who cooperated in the collection of the samples and supplied the data on soil type, organic matter, and hydrogen-ion concentration. The latter were determined by the quinhydrone method.

[3] Because of the necessity of delay in plating, a parallel study of the effect of holding a sample in cold storage was undertaken. It was found that, when a sample was kept air-tight (in cold storage) and opened for plating at the end of 7, 14, and 55 days and for longer periods, the total number of organisms and the percentage of bacteria increased up to 14 days' storage and thereafter decreased, while the reverse was true of Actinomyces and other fungi.

It is realized that one sampling of a soil does not give a very clear picture of the occurrence or activities of the microflora. It was thought, however, that some evidence might be obtained regarding the relative prevalence of the 3 large groups of soil microorganisms and a possible indication of the relation of their occurrence to the amount of disease. Because of the amount of labor involved it was found impossible to plate out all samples, 231 samples from 77 fields were plated. Waksman's egg-albumen agar was used for determinations of numbers of bacteria and Actinomyces, and Waksman's acid fungus agar for counts of fungi. Ten plates of each agar were used for each of the 3 samples from each field.

Soil moisture determinations were made on each sample at the time of plating. The data given in the tables are on a dry weight basis.

In addition to the samples obtained from the potato fields, 9 samples were also taken from virgin soil identical in type to the potato fields adjacent.

RESULTS

Prevalence of the two Diseases. Scab was of common occurrence throughout the entire area surveyed and was quite severe in many fields. There were no fields where the potatoes were entirely free from either *slight* or *medium* scab, and *severe* scab was present in 67 per cent of the fields. The infection in all samples averaged 10.5 per cent *slight*, 12.5 per cent *medium*, and 1.4 per cent *severe* scab, with an additional 30 per cent of a superficial or russet type of scab. The percentage of scab severe enough to require sorting out is best approximated by combining the *medium* and *severe* scab. There were 75 fields with less than 10 per cent of combined *medium* and *severe* scab, 21 fields with 10 to 25 per cent, 12 fields with 25 to 50 per cent, and 7 fields with over 50 per cent. The occurrence of potato scab in this area is clearly shown to be of major importance and, primarily, a problem of soil infection.

The data on Fusarium wilt showed this disease to be almost as prevalent as scab. There were only 7 of the 118[4] samples of potatoes that were entirely free of stem-end rot or vascular discoloration. The average amount of infection for all fields was 4.5 per cent with about half, or 2.4 per cent of this being of a severe type. There were 9 samples with 10 to 20 per cent, and one each with 30, 33, and 47 per cent. The latter was from a field that had been under cultivation for only 9 years and had never before been planted to potatoes. The sample containing 30 per cent infection was grown on virgin soil. The type of infection in the tubers was chiefly a vascular discoloration and half of this was of a severe type and occurred

[4] A few of the fields were divided into two samples because of differences in cultural practices, which accounts for the 18 samples in excess of the 100 originally planned.

as wide bands of discolored tissue extending more than one-fourth of the length of the tuber. There was very little stem-end rot without an accompanying vascular discoloration and in the combined form the severe types predominated.

The importance of the Fusarium-wilt problem is not fully indicated by the above data. It must be remembered that most of these infected fields contained wilted plants in the field and that many of these plants wilted early and did not produce tubers. The disease unquestionably resulted in reduced yields and more unmarketable tubers but in this survey it was not possible to keep a record of wilted plants in the field nor to obtain accurate yield records that would be comparable. Here, as with the scab, we have a problem that is primarily one of soil infection.

A summary of the commercial fields inspected for certification in this area the same year shows also the severity of these two diseases. Eighty-five per cent of the 530 fields inspected contained Fusarium wilt and these infected fields averaged 1.4 per cent, while 84 per cent of the lots of tubers showed infection in the bin inspection and averaged 2.5 per cent. It must be remembered also that diseased plants often were rogued out of the fields before inspection and that severely infected fields were disqualified before the bin inspection. Bin inspection showed that 73 per cent of the fields yielded scabby potatoes, averaging 10.1 per cent of infected tubers that would have to be sorted out of a U. S. No. 1 grade.

Effect of the Preceding Crop. The variation in the types of rotations or crop sequences used over a 5-year period was so great that only the crop immediately preceding potatoes was considered (Table 1). There was little difference in the amount of scab following wheat, oats, rye, and barley and these grains were all grouped together. There was more scab, both total and severe, following corn and summer fallow than following the small grains, with the legumes falling between these two groups. It must be remembered that there was considerable variation in each group as indicated by the probable errors in table 1 and that definite conclusions cannot be drawn in the groups containing only a few fields, as in the case with the legumes and summer fallow. There is, however, a clear indication that cultivation of fields the year preceding potatoes tends to increase scab as contrasted with noncultivated crops. The fact that no significant differences occurred with the different small grains and that summer fallow increased scab similarly to corn indicates that the cultivation rather than the crop itself was the significant factor.

In one field of oats the grower had burnt off the stubble on one-half of the field. The test row was placed with one-half on each part of the field. The oat stubble not burned produced 75 per cent total scab and 2.8 per cent Fusarium wilt in the tubers, while that part of the field with the

TABLE 1.—*The effect of the crop immediately preceding potatoes upon the occurrence of scab and Fusarium wilt[a] and upon the number and percentages of microorganisms.*

Crop preceding potatoes	Disease records				Microorganisms per gram of soil				
	Number of fields	Total scab per cent	Medium[b] and severe scab per cent	Fusarium per cent	Number of fields	Total number of organisms, (000 omitted)	Bacteria per cent	Actinomyces per cent	Fungi per cent
Small grains	59	51.2 ± 2.4[c]	13.1 ± 1.2	4.8	33	43,699	94.6	5.3	.07
Alfalfa or sweet clover	7	60.5 ± 4.1	12.2 ± 2.5	2.4	5	39,069	94.4	5.5	.11
Corn	18	62.1 ± 4.3	22.5 ± 4.7	3.9	8	40,667	93.8	6.1	.12
Fallow	3	80.0 ± 4.9	27.2 ± 9.2	3.0	2	36,392	92.7	7.2	.09
Virgin soil	1	67.0	4.3	29.5	.....	.....	.....	.....	.....

[a] All figures are based on the weight of infected tubers.
[b] For definition of scab types see p. 518.
[c] The probable error was determined by the following formula:

$$\sqrt{\frac{\Sigma(x^2)}{n} - \left[\frac{\Sigma(x)}{n}\right]^2} \times .6745 = PE_s.$$

$$\frac{PE_s}{\sqrt{n}} = \text{P. E. of mean.}$$

stubble burned off produced only 47 per cent scab and no wilt. The soil was very uniform and, other than for the burning, the field was handled as a unit, so that both plots received similar treatment.

In regard to Fusarium wilt there were few outstanding differences. The smallest amount of wilt occurred following the legumes. In the fields following small grains there was a wide variation in the percentage of wilt, indicating that factors other than the preceding crop were probably of great influence. There was nothing to substantiate the common opinion of many growers that wilt is worse on corn land.

The fields grouped according to table 1 failed to show any correlation between disease and either organic matter or H-ion concentration. Plate cultures from 48 of these fields showed a higher average percentage of Actinomyces in the fallow and cornfields than in the small grains. The largest number of Actinomyces was in the fallow soil. The higher percentage of Actinomyces was accompanied by a lower percentage of bacteria and a slightly higher percentage of fungi. The average total number of all 3 groups of organisms was least in the fallow and highest with the small grains.

Interval between Potato Crops. A common recommendation to potato growers is to allow a period of time, usually 4 or 5 years, to elapse between potato crops. In view of the fact that virgin soil produced a large amount of scab (Table 1) the data were examined to determine the effect of the length of time between crops of potatoes upon the amount of scab and Fusarium wilt.

None of the fields had been planted with potatoes during the previous 2 years, so it was impossible to determine the effect of a recent crop of potatoes. The data are shown in table 2.

TABLE 2.—*The effect of the interval between potato crops upon the amount of scab and Fusarium wilt*

Interval between potato crops	Number of fields	Total scab per cent	Medium and severe scab per cent	Fusarium per cent
2 to 4 years	18	78.5	31.4	3.4
More than 4 years	10	56.6	12.1	5.0
No potatoes	61	46.2	9.8	5.3

The highest percentage of scab was for the interval of 2 to 4 years. There was a sharp drop, however, between this group and the group with an interval longer than 4 years and a further reduction in those fields that had never been planted to potatoes.

These data show that while intervals of more than 4 years between potato crops tend to reduce scab, that time alone is not sufficient to eliminate the disease or even to prevent serious loss.

The above conclusions do not apply to Fusarium wilt. In fact they are apparently the reverse. As shown in table 1, one of the most severely infected fields was planted on virgin soil.

Effect of Manure and Legumes. It is often stated that the addition of manure increases the amount of scab and that the use of a green manure in the form of alfalfa will reduce scab.

In this survey only 6 fields had ever received an application of manure and none of these within 2 years. Two that had never been planted to potatoes or alfalfa produced 31 per cent infected tubers, while the other 4 that had grown potatoes previously, produced 85 per cent infection. Alfalfa preceded potatoes in 9 fields that had never been planted to potatoes or received manure, and these fields produced 41 per cent infected tubers. Fifty fields that had never been planted to either potatoes or alfalfa or received manure averaged 48 per cent infection of scab.

The effect of previous potato crops was more pronounced than the effect of either manure or alfalfa, but there was no indication in these few fields that the addition of manure in previous years had resulted in more scab but rather the reverse, as less scab occurred than following alfalfa. The number of fields involved, however, is too small to warrant definite conclusions.

Soil Reaction. It has frequently been stated in the literature that low pH values result in decreased scab and that a pH of about 5.0 to 5.4 is the desirable range for scab control and increased yield of marketable potatoes. This is considered to be of great importance in the eastern part of the United States where sulphur can be applied to the soil to bring about the desired acidity. Concerning the effect of increased alkalinity upon the occurrence of scab there is very little published evidence.

Most of the soils upon which potatoes are grown in western Nebraska are alkaline and the others are only slightly below the neutral point. The lowest pH value found in any of the soils tested in this survey was 5.92. All others were above 6.3 and 56 per cent were above 7.0 in the upper 6 inches. Table 3 shows the summarized data when the soils are grouped with each group having a range of 0.5 pH in the first 6 inches of soil. The figures for the second 6 inches were always slightly higher but still within the same pH group.

As might be expected in a survey of this type with so many uncontrolled factors, there was considerable variation in the fields in each group, as is shown in the probable errors. There was, however, a very evident and consistent decrease in both the amount and severity of scab as the pH values increased from 5.92 to 8.25. This result was exactly the opposite from what might be anticipated on the basis of the literature.

TABLE 3.—*The relation of the reaction of the soil to the occurrence of scab and the number of microorganisms*

Groups based on pH of first six inches	Scab record			Microorganisms per gram of soil							
						Bacteria		Actinomyces		Fungi	
	Number of fields	Total scab per cent	Medium and severe scab per cent	Number of fields	Total number of organisms (000 omitted)	Number (000 omitted)	Per cent	Number (000 omitted)	Per cent	Number	Per cent
5.9–6.5	10	68.4 ± 5.72	16.4 ± 3.8	6	49,835	47,233	94.8	2,555 ± 121	5.2 ± 0.36	46,766	.09
6.5–7.0	29	56.8 ± 4.08	15.8 ± 2.7	13	37,254	34,750	93.3	2,465 ± 147	6.9 ± 0.33	39,033	.10
7.0–7.5	30	55.1 ± 3.30	15.5 ± 2.5	14	38,057	35,819	94.1	2,273 ± 160	6.2 ± 0.35	29,200	.07
7.5–8.0	13	48.0 ± 4.02	10.8 ± 1.8	9	47,400	45,340	95.6	2,031 ± 238	4.6 ± 0.54	29,465	.06
8.0–8.3	7	29.8 ± 5.49	7.9 ± 2.8	7	34,016	32,768	96.3	1,238 ± 92	4.1 ± 0.29	18,235	.05

As shown in table 3, the decrease in the amount of scab coincides with a decrease in both the total number and percentage of Actinomyces. There was no correlation between the total number of organisms and the occurrence of scab. There was no apparent relation between pH and the occurrence of Fusarium wilt.

Soil Type. The soils of 59 of the fields were classified in the Rosebud series and the remainder as Dunlap, Dawes, Valentine, Tripp, Epping, and Cheyenne. The number of fields in each of these latter were too small for purposes of series comparison. Twenty-eight of the fields were classified as silt loams and these averaged 67 per cent infection of scab. Twelve fields were loams and averaged 51 per cent infection, while 40 fields were very fine sandy loams and averaged 47 per cent infection. Samples plated from 12 silt loams, 9 loams, and 18 very fine sandy loams, chiefly from the Rosebud series, gave the following results in percentages of Actinomyces, 6.8, 5.0, 5.0, respectively. The total number or organisms was highest in the very fine sandy loams.

There was no correlation between the nitrogen content of the soil and either scab or wilt.

Time of Planting. Time of planting potatoes is known to be an important factor in influencing the development of scab in western Nebraska, late planted potatoes usually having the least scab due to the lower temperatures that prevail during tuber development. When the fields in this survey were grouped on the basis of date of planting, commencing June 1, the following average percentages of total scab were found for successive 10-day periods, 38.6, 51.9, 61.8, and 74.8 per cent. This reversal of the usual effect of late planting was probably due to the high rainfall during June and July, followed by dry weather in August and September, thus indicating that moisture may be more important than temperature in the development of scab. No correlation of date of planting and wilt was evident in these data, although observations and records over a period of years indicate that the same relationship holds as with scab.

Weather Conditions. There was no obvious correlation between either disease and temperature or rainfall as recorded by the U. S. Weather Bureau at 8 points in the area of the survey, or by the record of rainfall obtained by the use of rain gauges at 5 of the farms. The great variation in rainfall between fields only a few miles apart made it impossible to draw any conclusions from a comparison of large areas.

The rainfall at the 8 stations during June and July was above normal, the average rainfall being 3.71 and 3.45 inches, respectively. Only 2 stations reported less than 2 inches of rain in June and July. It was very dry during August and September, the average rainfall being 0.96 and 0.59 inches, respectively, with only 4 stations reporting over 1 inch in August and only 1 in September.

The mean monthly temperatures for 7 stations scattered through the survey area were as follows:—June, 59.0°; July, 70.9°; August, 70.1°; September, 59.7° F. The widest range occurring between these stations during any of the 4 months was 3 to 4° F. These temperatures were considerably below normal in June, slightly below in September, and about normal in July and August. In general, 1928, the year of this survey, was one of the worst scab years from 1925 to 1930.

Soil Moisture. The soil samples plated out varied from 8 to 32 per cent moisture. When all soils were grouped into 5 groups it was found that on a dry-weight basis the total number of organisms was higher in those few soils above 24 per cent. The percentage of bacteria was lowest and that of the Actinomyces was higher in the medium group, with the reverse occurring in the extremely dry or wet soils, while the percentage of fungi was higher in the wetter soils.

Virgin Soils. Comparative data from plate counts of virgin soils and adjoining cultivated soils were obtained from 9 fields and gave the following results:—average total organisms in virgin soils, 35,100,000 as against 31,900,000 for cultivated soils, bacteria 93.9 and 95.2 per cent, Actinomyces 6.2 and 4.8 per cent, and fungi .06 and .08 per cent, respectively. There was considerable variation, however, in the data from individual fields and the comparable virgin soils.

SUMMARY

A survey was made of the amount of infection of scab and Fusarium wilt from the soil in the high plains area of western Nebraska by planting portions of a lot of treated seed potatoes on each of 100 farms.

There were no fields entirely free from scab. The average percentage of infection of all fields was 10.5 per cent *slight*, 12.5 per cent *medium*, and 1.4 per cent *severe* scab with an additional 30 per cent of superficial scab.

Ninety-four per cent of the fields produced tubers with stem-end rot or vascular discoloration. The average for all fields was 4.5 per cent, with about one-half of this being of a severe type.

The following factors were correlated with a high percentage of scab infection: cultivated crops or summer fallow the preceding year, as contrasted with small grains, an interval of less than 4 years between potato crops, decreasing pH values of the soil from 8.25 to 5.92, silt-loam soils, as contrasted with loams or very fine sandy loams, and high number and percentages of Actinomyces in the soil.

No correlation was found in these fields between scab and content of organic matter, and rainfall or temperature, probably because of the greater influence of other limiting factors and the variability naturally occurring in a survey of this type.

There was no correlation between any of the factors recorded and the occurrence of Fusarium wilt as measured by stem-end rot or vascular discoloration except that less wilt occurred following legumes than after other crops.

The results here reported are considered of value chiefly in opening up further lines of inquiry, particularly as regards the effect of previous crops and of H-ion concentration of the soil, rather than as a practical recommendation for the control of these diseases. The results also clearly indicate that in the complicated field of soil-borne diseases conclusions drawn from observations or experiments in one section of the country do not necessarily hold for conditions in other sections.

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AN UNDESCRIBED SCLEROTIUM FUNGUS PREVALENT IN NORTHEAST TEXAS

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In September, 1932, following a period of wet weather, a fungus resembling *Phymatotrichum omnivorum* (Shear) Duggar was discovered at the United States Cotton Breeding Field Station, Greenville, Texas, growing on decayed cotton stalks and roots a few inches below the surface of the soil. It was first discovered in a field that had been subsoiled a few weeks earlier and brought to the attention of the writers by H. C. McNamara, Associate Agronomist and Superintendent of the Station. A few days later examinations of the various other plots revealed that it occurred not only on old fragments of cotton stalks and roots buried a few inches in the soil, but was rather abundant on the under surface of old cotton leaves which partly covered the soil. In many of the areas examined, the fungus had spread for some distance in the soil, forming abundant sclerotia as elongate swellings along mycelial strands (Fig. 1).

DESCRIPTION OF MYCELIA AND SCLEROTIA

The mycelium of the fungus as found on the decayed roots and in the soil is white when young, but becomes pale yellow and occasionally ochraceous buff with age. It is uniformly septate, and branching is either opposite or alternate just below the septa (Fig. 2), with individual hypha averaging 5.5 μ in diameter and 60 μ in length. A characteristic behavior of new cells arising from the side of the primary hypha is to grow in opposite directions. In some cases, the lower portion of the cell grows downward for a short distance (see figure 2 at point indicated by arrow) and forms new cells either in parallel fashion or at an angle of 45° to the main filament. As a result of anastomosing of filaments the strand hyphae are formed, which later differentiate into sclerotia in the form of elongate swelling similar to the cotton-root-rot fungus.

The sclerotia are white to pale yellow, depending on age, and vary considerably in size and shape. As a rule, they average about 1 mm. in width and 5 mm. in length, being round to ovoid, ellipsoid, variously constricted, and often forked at the strand connections (Fig. 3). The exterior of the sclerotia is smooth or glabrous, and cross sections stained in crystal violet reveal cellular structures similar to those of cotton-root-rot sclerotia, composed of anastomosed hyaline hyphae.

FIG. 1. Sclerotia and strands of an undescribed sclerotium fungus observed on decayed cotton roots, stalks, and leaves in the soil at Greenville, Texas. ×15.

CULTURAL STUDIES AND GROWTH ON MEDIA

The fungus is quite easily isolated and makes a rapid growth on a variety of culture media. One peculiar feature is that pure cultures are often obtained from sclerotia without subjecting the latter to previous surface sterilization with the usual toxic agents, such as mercuric chloride, alcohol, calcium hypochlorite, etc. On such media as carrot, corn-meal, and Dug-

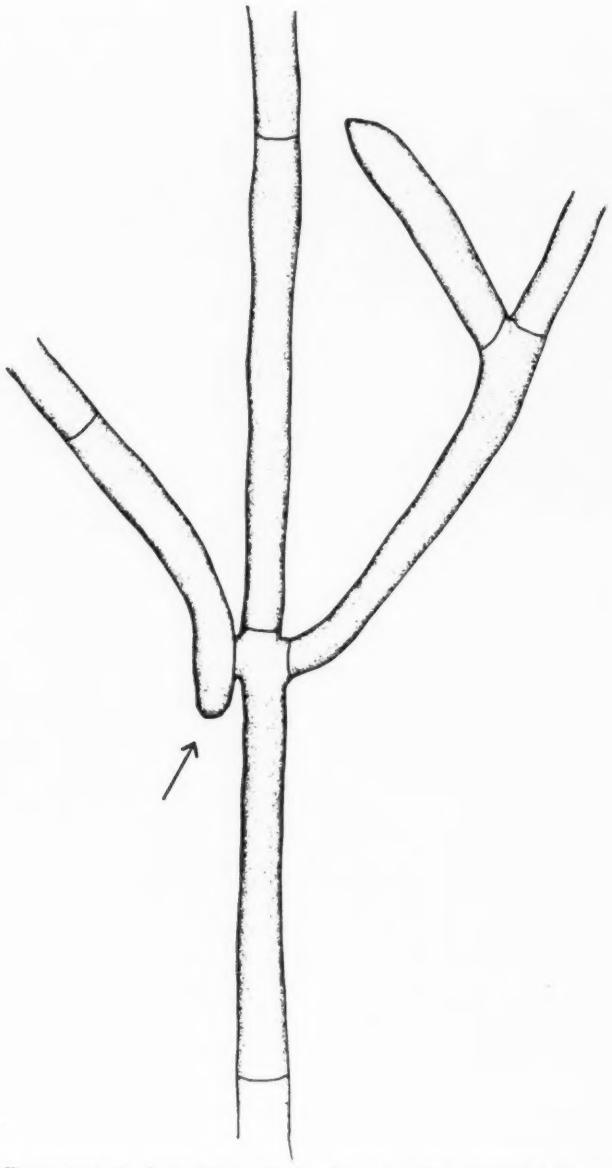

FIG. 2. Characteristic branching of the fungus after growing in a hanging-drop culture for 24 hours. Note septate mycelium and new hyphae arising just below the septa. Photographed from a camera-lucida drawing. ×840.

gar's synthetic agar, sclerotia are formed in large numbers at a temperature of 29° C. In hanging-drop cultures, new filaments arise from the surface cells of the sclerotium and from those of the connecting strands within 24 hours (Fig. 4). These filaments anastomose freely, and rapidly give rise

B

A

FIG. 3. Sclerotia and strands obtained from soil cultures of the fungus. A. Mass of sclerotia and strands from a soil culture 8 days old. ×15. B. Sclerotia from a soil culture 4½ months old. Note variation in size and shape and also strand connections. ×15.

to the arachnoid mycelium composing the strand. In quart-jar cultures of Wilson clay loam with a moisture content of 30 per cent and pH of 7 or 7.5, the strands ramify in the soil quickly and form sclerotia in large numbers within 3 or 4 days.

FIG. 4. A sclerotium with the connecting strand hyphae showing new filaments arising from the surface cells after being in a hanging-drop culture for 24 hours. ×135.

Features that distinguish the fungus from *Phymatotrichum omnivorum* are the following: finer texture of the strands; rapid formation of sclerotia on agar; apparent saprophytic habits; and the ease with which the fungus is cultured. Also, in contrast to the root-rot fungus, the mycelium does not have right-angle branches or acicular hyphae. Since no spore stages were observed in any of the cultures previously mentioned, which varied in age from a few days to over 3 months, the writers believe that this probably is

a sterile fungus similar to the Rhizoctonia, Sclerotium, and Ozonium groups. In attempting to place it satisfactorily in any of the above genera, some difficulties have been encountered; however, the character of the hyphae and the presence of plectenchymatic strands preceding the development of sclerotia, appear to justify placing this fungus tentatively in the genus Ozonium. The species in question, insofar as the writers have been able to determine, has not been encountered or described heretofore by mycologists. Therefore, for taxonomic convenience in distinguishing this fungus from the parasitic Ozonium (*Phymatotrichum omnivorum*) responsible for cotton root rot and also widely prevalent in Texas, the name *Ozonium texanum*, sp. nov., is proposed.[1] A description of the organism is appended.

Ozonium texanum, sp. nov.

Fructificatione ignota; mycelio sterile septato, juvenile et in culturis albido, senescente pallide flavescente-ochraceo, infra septa oppositer vel alterne ramoso; hyphis plerumque 5.5 μ diam. et 60 μ longis; in fasciculis plectenchymicis, elongata sclerotia formantibus, coalitis; hyphis novis saepe diversis vel ad angulos 45° ex filamentis crescentibus; sclerotiis albidis vel pallide flavidis, in magnitudine et forma variabilibus, plerumque 1 mm. latis, 5 mm. longis, interdum rotundis vel ovoideis, generaliter ellipsoideis, radiciformibus, varie constrictis, saepe ad connectiones fasciculorum furcatis.

In radicibus, caulibus et foliis putridis *Gossypii* in terra, Greenville, Texas.

No fructification definitely known. Sterile mycelium septate, white when young or growing in cultures, becoming pale yellow to buff with age. Branching opposite or alternate below the septa, with hyphae averaging 5.5 μ in diameter and 60 μ in length. New hyphae frequently growing in opposite directions or at an angle of 45° to the primary filament. Hyphae forming plectenchymatic strands, which enlarge into elongate sclerotia. Sclerotia white to pale yellow, variable in size and shape, averaging 1 mm. in width to 5 mm. in length, occasionally round to ovoid, but usually ellipsoid, root-like, variously constricted, and often forked at strand connections.

Hab. Hyphae and sclerotia on decayed cotton roots, stalks, and leaves in the soil at Greenville, Texas.

Cultures have been deposited in the Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
GREENVILLE, TEXAS.

[1] Neal, D. C., and R. E. Wester. An undescribed sclerotium fungus prevalent in northeast Texas. Phytopath. 23: 24. 1933.

A BACTERIAL CANKER OF PEAR TREES NEW TO CALIFORNIA

EDWARD E. WILSON

(Accepted for publication July 12, 1933)

In the autumn of 1931 a cankerous disease of Wilder pear branches was found in an orchard near Penryn, Placer County, California. A preliminary survey showed that the same type of canker was present on trees of the Beurre Bosc and Comet varieties; the Bartlett variety, on the other hand, being relatively free of the disease. Similar cankers have since been found in El Dorado, Sacramento, Lake, and Mendocino Counties on Easter Beurre, Winter Nellis and Beurre Hardy varieties of pears, and on an unknown variety of apple.

The external appearance of newly formed cankers is rather characteristic in that the periderm becomes loosened and raised (Fig. 1, D), portions of it sloughing away to expose the spongy, disorganized cortex beneath. In active cankers, the cortex is light tan or buff; brownish streaks may extend upward and downward through the cortex and outer phloem for a distance of several inches beyond the outwardly visible canker. During the summer, healing beneath the diseased area causes the outer bark to become longitudinally and transversely cracked (Fig. 1, A, B). At the same time the streaks at the apical margins of the cankers change from a light brown to a dark brown or almost black color (Fig. 1, C). Another characteristic feature of the cankers is their confinement to the cortex and outer phloem. Thus, while the disease may eventually kill limbs or entire trees, it does so rather slowly in average years. In the spring of 1933, on the other hand, a number of pear trees were killed in Placer County. Some of these had been diseased for several years, others had only recently become so.

Field diagnosis of this canker may be difficult in orchards where fire blight is present and it seems probable that these diseases have often been confused. It is desirable, therefore, to point out some of their contrasting symptoms.

Period of Activity of Disease. The new disease is active in the cool weather of late autumn, winter, and early spring. Fire blight is most active in the warm weather of late spring and summer.

Tissues Involved by the Disease. The disease is primarily one of the outer bark, although it may in certain years, such as 1933, involve all of the bark. Fire blight, although first involving the cortex, eventually extends to the cambium.

External Appearance of Cankers. The cankers of the new disease are first visible as light brown irregular patches on the limbs, the brownish

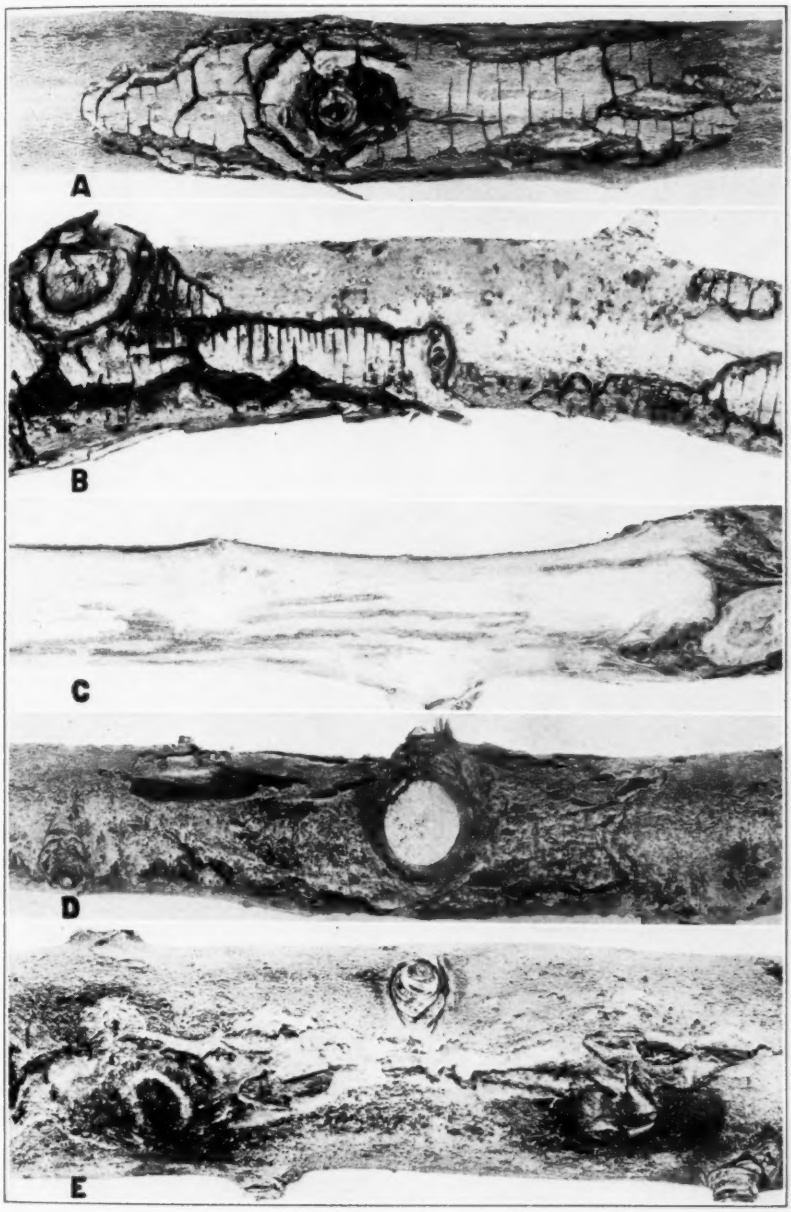

FIG. 1. A–D. Cankers from natural infection. A. Canker surrounding a lateral spur. B. Longitudinal and transverse cracking of bark over diseased areas. C. Black streaks extending through the outer bark of an infected branch (periderm removed). D. Active canker originating at a pruning wound. Periderm is loose and beginning to slough away. E. Symptoms produced by inoculating a Wilder pear branch.

color being due to the loosened periderm. Later the periderm may wholly or partially slough away, exposing the spongy cortex. Cankers of fire blight, on the other hand, are dark grey to black and the periderm usually remains firmly attached.

Internal Appearance of Cankers. Bark affected by the new disease is spongy and of a tan or buff color. Numerous fine brown streaks often extend from the apical margins of affected areas into otherwise apparently healthy bark. In fire-blight cankers the diseased tissue is first water-soaked; later, a distinct reddish-brown color appears. The cortex remains firm; streaks, if present, are few in number.

Isolations from the cankers of the cortical disease yielded bacteria consistently. In comparatively inactive cankers the best results were obtained by removing material for isolation from the zone of streaks at the lower or upper canker margins. The organisms from different sources, although differing in chromogenic characters on potato-dextrose agar, were on the whole similar.

Inoculation experiments, conducted during the winters of 1931–32 and 1932–33, showed that the bacteria were capable of producing symptoms on pear branches in every way similar to those in nature (Fig. 1, E). Organisms similar to those used in the inoculation work were obtained upon reisolation, and these bacteria in turn produced cankers when inoculated into pear branches. In order to guard against the inclusion of *Bacillus amylovorus* in the inoculum or its entry into the wounds after inoculation, the cultures were punctured into green pear fruits. The bacteria obtained in the reisolations from limb cankers were also inoculated into green pears. In no case did the characteristic fire-blight symptoms develop on the fruit. An additional guard against infection by *B. amylovorus* was afforded by the numerous control inoculations. In only 3 cases out of several hundred did the control punctures develop symptoms; these being those of the new disease.

A description of the causal organism is not given at this time, inasmuch as cultural studies are not complete and comparisons with certain other organisms are desirable. The bacterium differs from *Bacillus amylovorus*, both in type of symptoms produced on the host and in cultural characters. For example, it produces a greenish pigment on many media, while *B. amylovorus* produces no pigment. In this feature it allies itself with certain earlier named organisms. For example, the inoculation work indicated that it is similar to if not identical with the organism, *Pseudomonas cerasi*, producing cankers in stone-fruit trees. The writer has earlier

shown (4) that the stone-fruit organism[1] is very similar to *Pseudomonas prunicola*, reported by Wormald (7) as the cause of a green-shoot blight of plums in England. An organism described as *Phytomonas utiformica* by Clara (1) in New York is being compared with the California pear organism. Thus far the 2 organisms have proved similar.

Rosen (2) in 1932 mentioned a bacterial blossom and twig blight of pears and apples, the organism of which was similar to *Pseudomonas prunicola*. Later, (3) he made pathological and serological comparisons of a number of organisms, including his pear pathogen, *P. prunicola*, *Bacterium syringae*, and *B. citriputeale*, and found them to be indistinguishable.

A blossom blight of pears, differing from that produced by *Bacillus amylovorus*, has been found in California by Harvey E. Thomas. The organism obtained from such symptoms appears similar to those found in the cankers, described herein. Thus far, however, no detailed pathogenicity comparisons have been made of bacteria from the 2 sources. Such comparisons are planned and will be made the subject of a paper by Dr. Thomas.

Obviously, the organism mentioned herein cannot be assigned a name until it is compared with other similar organisms. It is becoming more and more evident that new species created from studies of the disease of a single host are often unjustified.

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[1] In his earlier publication (4) the writer stated that two chromogenic types of the stone-fruit organism existed. Later (5) he reported that one of these was considered to be *Pseudomonas cerasi*, the other a variety of the same species. A more recent article (6) deals with these types or varieties of *P. cerasi*.

STEM CANKER OF HOLLYHOCK CAUSED BY SCLEROTINIA SCLEROTIORUM[1]

PAUL A. YOUNG

(Accepted for publication August 7, 1933)

White and retted cankers were found in many stems of hollyhock, *Althaea rosea* Cav., in a garden in Bozeman, Montana, on October 17, 1931. This stem canker was studied during 1931 to 1933 because it resembled sunflower wilt, and because the hollyhock is a valuable ornamental plant.

REVIEW OF LITERATURE

Smith[2] described the stem canker of hollyhock. He published drawings of sclerotia in stems, and of sclerotia bearing immature and mature apothecia, named *Peziza sclerotiorum*. Richardson[3] described the stem canker of hollyhock and reported successful inoculations with a species of Sclerotinia. Young[4] described the Sclerotinia stem canker of hollyhock in Montana. Young and Morris[5] described the similar symptoms of Sclerotinia wilt of sunflower.

EXPERIMENTAL RESULTS

Symptoms and Signs in Hollyhock. Black to dark green, water-soaked, rapidly enlarging cankers appeared in the cortical tissues near the wounds in artificially inoculated hollyhock stems. The centers of the cankers dried and became white, with black or dark green margins. Most of the cankers girdled the stems within 2 to 4 weeks. Later, the leaves wilted on the stems above the cankers.

Mature cankers from natural and artificial inoculations were 5 to 45 cm. long, and had black, brown, or indefinite margins. Black and brown streaks appeared in the epidermis and xylem from 1 to 15 cm. above some cankers. Brown cankers developed on some tap roots attached to stems with basal cankers.

The epidermis of mature cankers was white or light brown (Fig. 1, A). Many cankers had 2 to many, nearly concentric, brown arc lines with di-

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper 37, Journal Series. The writer wishes to thank Professor H. E. Morris for valuable suggestions.

[2] Smith, W. G. New disease of hollyhocks. Gard. Chron. III, **8**: 324. 1890; and III, **9**: 791–792. 1891.

[3] Richardson, J. K. *Sclerotinia* disease of hollyhock, *Althaea rosea* Cav. Canada Dept. Agr. Div. Bot. Rpt. **1926**: 48–50. 1927.

[4] Young, Paul A. *Sclerotinia* stem canker of hollyhock. Phytopath. **23**: 39. 1933.

[5] Young, P. A., and H. E. Morris. *Sclerotinia* wilt of sunflowers. Mont. Agr. Exp. Sta. Bul. 208. 1927.

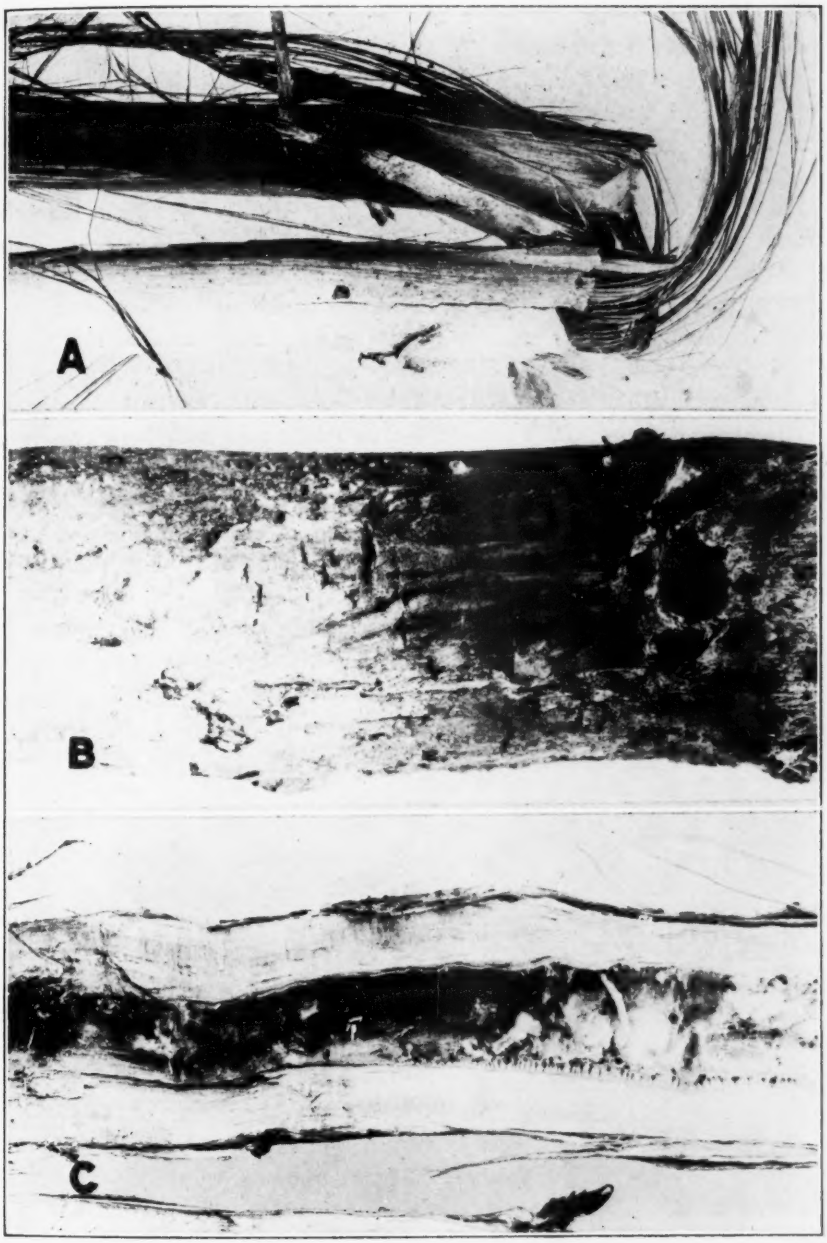

FIG. 1. Hollyhock stems naturally infected with *Sclerotinia sclerotiorum*. A. Canker with white, cracked epidermis and retted, protruding bast fibers. A sclerotium is shown. ×1. B. Canker with white epidermis and black sclerotia. ×2. C. Hollowed stem showing mycelium. ×1.3.

mensions of 3 to 15 by 0.3 to 1 mm. Some of these are lines marked the tops of epidermal wrinkles.

Hyphae grew below and in the epidermis, and emerged as white, conical pimples 0.3 to 1 mm. wide in the epidermis (Fig. 1, B). The pimples consisted of hyphae and epidermal cells. Large masses of hyphae emerged through the epidermis and formed black sclerotia (Fig. 1, B).

The dried epidermis remained intact on the cankers during a month or longer. Later, the epidermis on many cankers ruptured and released the retted bast fibers, which protruded from the cankers (Fig. 1, A, C).

This fungus decomposed the pith in the cankered parts of stems and formed dense masses of mycelium and sclerotia in the cavities thus formed (Fig. 1, C). It caused chambered pith in some hollyhock stems, resembling the chambered pith of *Juglans nigra* L.

The cankers caused by *Sclerotinia sclerotiorum* in hollyhock closely resembled the cankers caused by this fungus in sunflower.

Thick layers of white hyphae with sclerotia developed on the bases of many cankered, dead hollyhock stems in the garden during February to April, 1932. *Sclerotinia sclerotiorum* was isolated from one of these stems, and was used in the inoculations.

Inoculation of Hollyhocks. Two hundred hollyhocks, 1 to 3 years old, were transplanted on April 11, 1932, to a field where they grew normally. Blocks of agar bearing the *Sclerotinia sclerotiorum* isolated from hollyhock were placed in wounds in many of these living hollyhock stems and the wounds were covered with wet cotton. These stems were inoculated on July 16, 1932, and Sept. 1, 1932.

Cankers developed in 71 of these 75 inoculated stems within 13 days and killed the stems within 30 to 60 days. These cankers were essentially identical with the cankers from natural infections in the garden.

Evidence of Resistance. Cankers appeared only in the artificially inoculated hollyhocks in this field. The roots of these cankered plants apparently were not attacked by the fungus within 4 months. Evidently, hollyhocks are more resistant to *Sclerotinia sclerotiorum* than are sunflowers, because this fungus usually kills sunflower roots and commonly spreads to neighboring plants.

Inoculation of Sunflower (*Helianthus annuus* L. var. White Beauty). Blocks of agar bearing the Sclerotinia isolated from hollyhock were placed in wounds in large, living sunflower stems, and the wounds were covered with wet cotton. These inoculations were made on July 16, 1932, and Sept. 1, 1932. Cankers developed in 18 of the 21 inoculated stems within 5 to 13 days, and quickly rotted the cortical parenchyma tissues. The Sclerotinia spread into the roots of the infected stems and killed the plants within 60 days.

An inoculated sunflower head developed a canker that rotted the parenchyma tissues in 2/3 of the head within 13 days. This canker bore 4 large sclerotia in the spongy part and many thin flat sclerotia in the seed layer within 39 days.

These cankers in the stems and head were like those described by Young and Morris.[6]

Reisolations and Cultural Characters of Sclerotinia sclerotiorum. Isolations from 3 cankers in hollyhock stems and from 1 canker in a sunflower stem all gave pure cultures of a Sclerotinia identical with the cultures used in inoculating these stems. All of these cultures produced abundant white mycelium fuzzy white immature sclerotia bearing hyaline droplets of liquid, and numerous mature black sclerotia with dimensions of 1 to 10 by 1 to 5 mm. These characters are typical of *Sclerotinia sclerotiorum.*

Germination of the Sclerotia. Sclerotia and mycelium with attached pieces of agar were cut from pure cultures of *Sclerotinia sclerotiorum* and were planted in the soil in 6 flower pots in the greenhouse. These agar cultures were transfers of the cultures used in inoculating the hollyhocks and sunflowers. The sclerotia grown on agar produced 11 mature apothecia and 3 filiform bodies within 94 to 173 days in 4 of the pots.

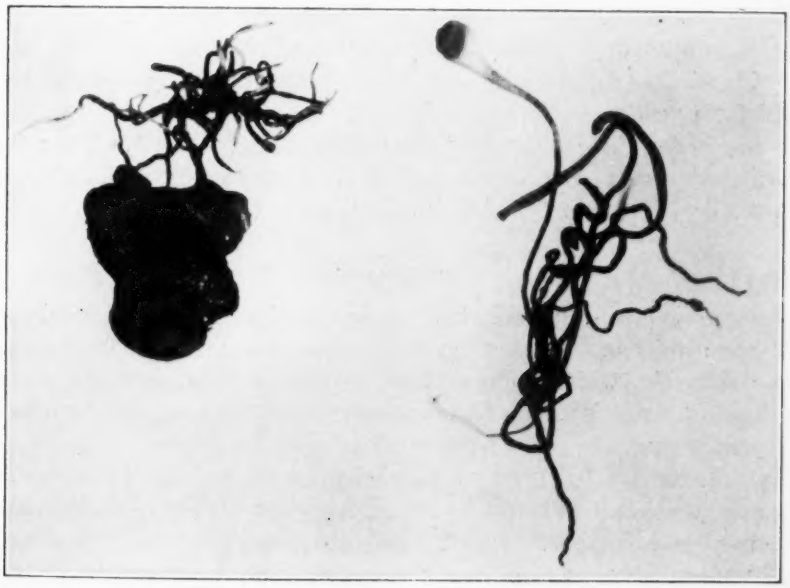

FIG. 2. Filiform bodies and a clavate, atypic apothecium of *Sclerotinia sclerotiorum* produced within 52 days by sclerotia from a sunflower root. ×1.8.

[6] See footnote 5.

Sclerotia from the cankers in the stems of hollyhocks and sunflowers were planted in the soil in flower pots. These cankers were caused by *Sclerotinia sclerotiorum* in the artificial inoculations. The sclerotia grown inside hollyhock stems produced 4 mature apothecia and 1 filiform body within 115 to 141 days in 3 of the 28 pots. The sclerotia grown on sunflower roots and inside sunflower stems produced 2 mature apothecia and 15 filiform bodies (Fig. 2) within 52 to 100 days in 4 of the 9 pots. Mature apothecia were illustrated by Young and Morris,[7] who described the method used in germinating sclerotia.

The apothecia were 1 to 5 mm. wide and avellaneous brown. Their stipes were 5 to 20 mm. long, with black to dark brown lower parts and light brown upper parts.

The filiform bodies (Fig. 2) were immature apothecia, and were black to dark brown, like the bases of apothecial stipes. Some of the filiform bodies produced mature apothecia, but others produced only light brown tips that did not become apothecia. Many of these filiform bodies remained unchanged in shape and color and did not form apothecia.

One of the sclerotia grown on agar produced an apothecium 5 mm. in diameter. Fragments of it studied in distilled water revealed asci 108 to 144 by 10 microns, with 8 spores per ascus; ascospores 9 to 12 by 5 to 7 microns; and clavulate paraphyses 81 to 100 by 3 microns. Iodine in an aqueous solution of potassium iodide made the tips of the asci blue. Essentially identical asci and spores were found in the similar apothecia produced by sclerotia grown inside cankers in hollyhock stems.

Name of the Fungus Causing Stem Canker of Hollyhock. The morphological characters of the fungus causing stem canker of hollyhock all agree with the description of *Sclerotinia sclerotiorum* (Lib.) deBary.

SUMMARY

Sclerotinia stem-cankers in hollyhocks exhibited the following symptoms and signs: white or light brown cankers 5 to 45 cm. long and girdling the stems; sclerotia, white pimples, and brown arc-lines on many cankers; retted bast fibers projecting from cankers; and sclerotia in stems made hollow by the fungus.

The fungus isolated from hollyhock was inoculated into hollyhock stems in which it produced symptoms and signs essentially like those resulting from natural infections. This fungus also was inoculated into sunflower stems in which it produced symptoms and signs identical with those of Sclerotinia wilt of sunflower. The fungus reisolated from these cankers in

[7] See footnote 5.

hollyhock and sunflower stems was identical with the fungus used in inoculating these stems.

Sclerotia grown in pure cultures on agar produced apothecia and filiform bodies. Sclerotia grown in cankers in hollyhock and sunflower stems also produced apothecia and filiform bodies. The filiform bodies were immature apothecia.

Morphological characters showed that the fungus causing stem canker of hollyhock is *Sclerotinia sclerotiorum* (Lib.) deBary.

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ABNORMAL GERMINATION IN DUSTED WHEAT[1]

WILLARD CROSIER

(Accepted for publication July 14, 1933)

The injury to cereals occasioned through treatment with various dips, formaldehyde in particular, has led gradually to their abandonment in favor of the fungicidal dusts. In general the dust treatments, if made with reasonable care, have not resulted in injury, and reports of increased field stands, which may indicate increased percentages of germination, are not uncommon. Lambert *et al.*[2] determined the efficiency of various dusts and liquids in controlling the covered smuts of small grains. They found no indication of germination abnormalities with any dust, although applications of formaldehyde caused marked seed injury. Briggs[3] experimented with 24 commercially compounded dusts and copper carbonate, applying them at rates of 1 to 4 ounces per bushel. He states that no seed injury was encountered and that several cases of increased field stands were noticed.

In July of 1932 a sample of Marquis spring wheat was received by the Seed Laboratory for germination tests. The gray color of the seed, as a whole, as well as dust deposits on the brush end, were evidences that the sample had been drawn from dusted seed. Triplicate sets of 100 seeds each were placed to germinate at 18–20° C. Counts made after a 5-day germination period showed that the seedlings were abnormal in respect to strength and type of sprouts (Fig. 1). The average percentages were: normal seedlings 49; nearly normal plumules but short thickened roots 13; very abnormal plumules and roots 34 and dead seeds 4. With several seedlings the roots were clubbed and thickened to about 4 times the normal diameter and were only a fraction of the normal length. The germination tests were continued for 10 days longer in the expectation that the deformed seedlings would become more nearly normal. However, only 6 of the slightly abnormal seedlings developed into normal ones. From all indications this sample would not produce a satisfactory field stand. This was confirmed later by the vendor who stated that poor stands occurred in Maine and that the Experiment Station at Orono reported an unsatisfactory field stand in its plots.

[1] Published as Journal Paper No. 1 with the approval of the Director of the New York State Agricultural Experiment Station.

[2] Lambert, E. B., H. A. Rodenhiser and H. H. Flor. The effectiveness of various fungicides in controlling the covered smuts of small grains. Phytopath. **16**: 393–412. 1926.

[3] Briggs, Fred N. Seed treatments for the control of bunt of wheat. Phytopath. **16**: 829–842. 1926.

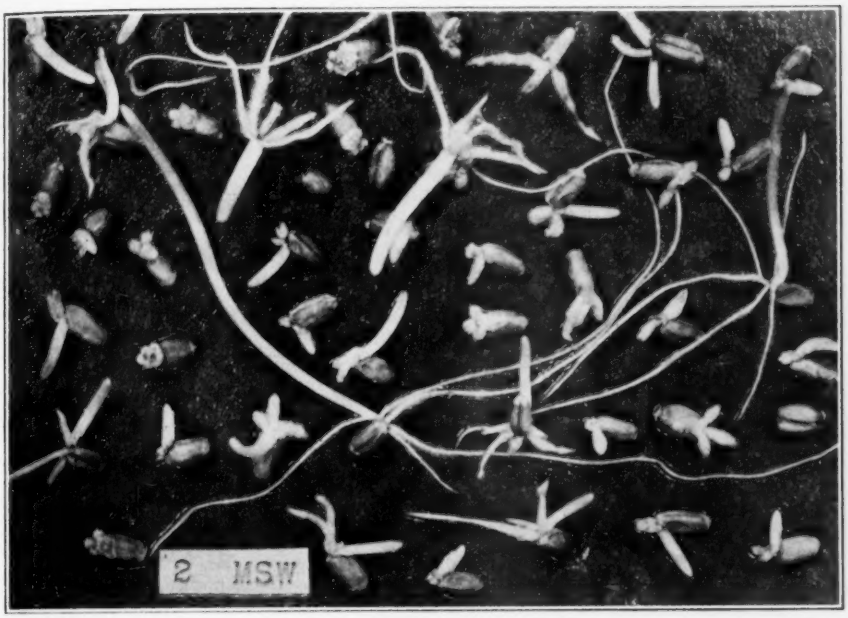

FIG. 1. Seedlings of Ceresan-dusted Marquis spring wheat after a germination period of 5 days.

The seed had been treated with Ceresan in March, 1931, was thoroughly dry at the time of the dust application, and had been held in cool, dry storage until the spring of 1932. No record of the field performance in 1931 was obtained. However, the vendor concluded from germination tests that the seed would produce normal field stands. Other seed lots, similarly treated and stored, were normal in both laboratory tests and field performance.

Additional laboratory germination tests were performed in November, 1932, or about 4 months after the first tests. In 10 lots of 100 seeds each, subjected to slightly different temperature and moisture relations, the percentages of normal germination varied from 54 to 33, the average of the entire 1000 seeds being 49. Washing the seeds in distilled water, brushing to remove the adhering dust deposits, or soaking in well-aerated running water for several hours had no effect on the type or rapidity of germination. The sample was tested the third time in February. The germination was read in 6 and in 16 days. In every lot a few seedlings placed in the "Nearly normal plumules but abnormal roots" list at 6 days were advanced to the "Normal" list at 16 days. None of the decidedly abnormal seedlings improved during this period. A comparison of these germination percentages appears in table 1.

TABLE 1.—*Percentages of germination in Marquis spring wheat dusted with Ceresan*

Date of test	Length of test in days	Condition of seedlings			
		Normal	Abnormal roots and nearly normal plumules	Abnormal plumules and roots	Dead seeds
1932	Growing season		Very unsatisfactory field stand		
July, 1932	5	49	13	34	4
July, 1932	15	55	7	34	4
November, 1932	5	49	14	30	7
February, 1933	6	52	16	26	6
February, 1933	16	60	8	26	6

The vendor's declaration that the orthodox method of treatment had been followed led naturally to the probability of other causes for this abnormal germination. Several hundred seeds were examined minutely under a binocular microscope. The seed coats were cracked or chipped in some grains so that the embryo was poorly protected. The percentages of injured and noninjured seeds were: badly cracked coats 4.6, chipped coats 46.4, and coats intact 49. It is not impossible that such a condition arose from vigorous mixing of the seed during treatment. A comparison of the germination of the seeds so classified is made in table 2.

TABLE 2.—*Percentages of germination in injured and noninjured wheat seed dusted with Ceresan*

Condition of seed coats	Variety	Normal sprouts	Abnormal roots nearly normal plumules	Very abnormal roots and plumules	Dead seeds
Cracked	Marquis spring wheat	9	17	71	3
Chipped		21	5	67	7
Intact		69	15	13	3
Chipped	Honor winter wheat	61	10	5	24
Intact		99	0	0	1
Bulk		96	1	0	3

Unfortunately, this entire lot of wheat had been treated so it was impossible to ascertain whether the seed-coat injury should be attributed to the mechanics associated with the dusting or to other causes. This also made it impossible to compare the germination of the dusted wheat with that of nondusted wheat of the same lot.

To see if this condition was at all common a sample of farmer-treated

(copper carbonate) seed was examined and placed to germinate. This sample had been stored for over a year in a warm laboratory. Of 1000 seeds examined, cracking or chipping was found in 81. Germination results are recorded in table 2. The abnormal seedlings were closely comparable to those observed in the sample of Marquis. Chipped seed coats also were found in several nontreated lots of wheat. However, even after a year's storage, there was no appreciable difference in the germination of the seeds with chipped coats and with coats intact.

Attempts to induce abnormal germination by chipping the seed coats and dusting with Ceresan were unsuccessful. Seeds that had been severely bruised, chipped, or cracked and then liberally dusted were stored in tight containers for 3 months. No diminution in germination was observed. On the other hand, seeds treated while moist germinated very poorly after 3 months' storage. In most cases very few sprouts could be found.

It would seem that the question of seed treatment is in need of further consideration. Some attention also might be directed justifiably toward safe methods of treatment and subsequent storage for large-quantity trade lots of cereals.

SUMMARY

Abnormal laboratory germination has been observed in a sample of dusted Marquis wheat. It is thought that the dust injury to the seed was accentuated either by chipping of the seed coats or by faulty storage conditions.

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VARIABILITY OF PSEUDOMONAS CERASI IN PHYSICAL CHARACTERISTICS OF GROWTH ON SOLID MEDIA

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The shape, size, color, elevation, and consistency of the colony have long been considered important features in identifying bacteria. That is to say, a bacterial colony is reported to be flat or raised, of a butyrous, slimy, or gelatinous consistency and of a white or yellow color. The investigator may, early in the work, conclude that certain of these features are constant for his organism. In isolations from diseased tissue, he will select those colonies that conform to these characters and discard all others. This practice delays the recognition of variations within the same species, unless such variations are frequent in pure culture.

Studies of a canker of stone-fruit trees in California have revealed that not only does the pathogen, *Pseudomonas cerasi* Griffin, vary in pigment production,[1] but more recently the organism has been found to undergo, at times, a change in physical characteristics of the colony. Isolations from the cankers have occasionally yielded colonies very different from the more common type in elevation, topography, and consistency, whereas the colonies of the common type on potato-dextrose agar are rather flat, thin-margined, smooth-surfaced and of a butyrous consistency (Fig. 1, A); those of the variant are raised, with convolute surfaces and of a gelatinous consistency. Transfers of such colonies to potato dextrose agar slants resulted in a raised, convolute, gelatinous growth (Fig. 1, C). This type of growth persisted in certain cases for several months, although it was noted that in all cases the gelatinous character would eventually be lost in subsequent transfers and the flat, smooth-surfaced, butyrous growth appear instead.

Had not the colonies of the gelatinous type produced pigment similar to that formed by the typical *Pseudomonas cerasi*, they probably would have been regarded as those of an unrelated organism. Later evidence strengthened the conviction that the *Ps. cerasi* actually produced 2 distinct colony types. A culture of *Ps. cerasi* that had been subjected to the usual purification procedure a number of times was seen to change from the flat, butyrous condition into the raised, gelatinous condition. The first indication of such a change was the appearance of small, hemispherical pustules at dif-

[1] The existence of two chromogenic types of bacteria capable of producing identical symptoms was reported (5) in 1931. At the Atlantic City meetings the writer (6) stated that one of these types is considered to be *Pseudomonas cerasi;* the other a strain of the same species. Recently (7) these two strains have been described in more detail.

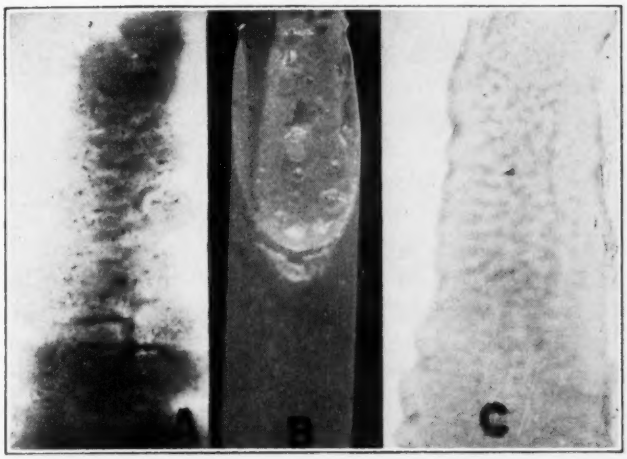

FIG. 1. Variation of *Ps. cerasi* in culture. A. Typical flat, thin-edged growth of butyrous consistency. Photographed by transmitted light. B. Pustules beginning to appear at different places in a typical flat streak of the organism. C. Raised, spongy, gelatinous growth produced by bacteria transferred from pustules of B. Photographed by transmitted light.

ferent places in the streak (Fig. 1, B). These pustules enlarged until the entire surface of the culture was covered by a gelatinous growth with a convolute surface. By plating out the bacteria from both types of growth in the same culture, it was found, in a limited number of trials, that the bacteria from the pustules produced only gelatinous colonies, while those from the flat areas produced both gelatinous and butyrous colonies.

Recently a single-cell culture of *Pseudomonas cerasi* changed from the butyrous into the gelatinous type. It has been possible to make a limited study of this variant. Earlier attempts at comparisons of the two types had resulted in inconclusive results, due to the reversion of the variant to the parent type before the studies were completed. In the case of the single-cell culture, however, the variant was maintained in culture long enough to conduct a number of inoculation and cultural experiments. These experiments showed that the variant was pathogenic, although it did not produce as extensive cankers as did the typical *Ps. cerasi*. The cultural features, as far as studied, were practically the same for both types. The variant did not appear to produce as much acid on sucrose and glycerin as did the parent type, but on 18 other carbon compounds no consistent differences could be found.

Although this phenomenon has not been studied sufficiently to warrant a conclusion as to its nature, a comparison of figure 1, B with photographs published by Hadley (4, plate 1) will show a striking resemblance between the pustules produced by *Pseudomonas cerasi* and secondary or daughter

colonies formed by *Vibrio cholerae* and *Bacillus typhosus*. Hadley (4, page 58) has the following to say concerning daughter colonies: "We have noted in many instances that the ability of a colony to generate papillae or daughter colonies seems invariably to be correlated with reactions of dissociative significance." Whether the variation reported herein is a manifestation of dissociation remains to be proved.

The occurrence of the variation affords a vein for speculation on the possible relationship between *Pseudomonas cerasi* and an earlier described canker-producing organism, *Ps. spongiosa* Aderhold and Ruhland (1, 2). In describing *Ps. cerasi*, Griffin (3) called attention to Aderhold and Ruhland's organism, and, although he considered the two similar, stated that he had never observed *Ps. cerasi* producing the "spongy" colonies attributed to *Ps. spongiosa*. Aderhold and Ruhland may have had reference to the gelatinous, convolute-colony characters similar to those mentioned herein. To say that nothing further stands in the way of considering the 2 organisms identical, would be unwarranted, since their descriptions differ in other details. *Ps. spongiosa* will remain in the literature as an inadequately described species until these differences can be harmonized or the organism is shown to be a distinct species.

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PHYTOPATHOLOGICAL NOTES

Further Observations on the Natural Distribution of the Cotton Root-rot Fungus.—The natural distribution of the fungus (*Phymatotrichum omnivorum* (Shear) Duggar), the cause of a cotton root rot, extends to the desert regions of the Southwestern States, as indicated in the report of an occurrence of the fungus on the roots of several species of desert plants near Florence, Arizona.[1] Later observations have extended to several other districts, and the fungus has been found on the roots of two species of paloverde and other prominent desert plants.

It now appears probable that the root-rot fungus will be found indigenous to most of the desert region included in the drainage basins of the Rio Grande, Gila, and Colorado Rivers.

The disease has been recognized recently in southern Utah, which may be considered provisionally as the northern limit of the fungus, while on the south it extends into Sonora and Lower California.

The *Phymatotrichum omnivorum* was found in Oct., 1933, on dead and dying plants of the Mexican poppy *Argemone* sp., a short distance south of the international boundary in a mountain wash that drains through the settlement at Jacumba, Calif., where an infestation had been found in some alfalfa fields in Sept., 1932.

The conditions of natural occurrence in the desert differ widely from those in the cultivated fields, where the ravages of the disease have attracted attention. Under the usual dry conditions the fungus does not appear on the surface, and the scattered desert plants may not be killed by the disease, even where many of the roots are found to be affected.

Only by the chance find of a dead plant, or by the spore mats coming to the surface in wet weather, can the fungus be detected. In the Gila Valley the infestations in the earliest susceptible crops often are traced to infected mesquite roots or stumps or the shrub Lycium, commonly known as "squawberry." In some cases, however, other native forms apparently are "carriers" of infection to cultivated plants. In addition to the native species listed in the previous paper[1] as root-rot carriers, the mycelium of the fungus was found, in 1933, on the roots of *Franseria confertiflora, Gutierrezia lucida, Platanus wrightii, Parkinsonia aculeata, Aster spinosa,* and *Cercidium torreyanum.*

Discovery of infestations on native plants in these widely separated areas makes it apparent that the fungus has there a natural distribution. Little is now known of the factors that influence its distribution. Recent

[1] Science, January 8, 1932, page 48.

evidence obtained by the writers indicates that the movement of water in erosion and drainage may play an important part. Infestations in the cultivated areas of Arizona and southern California are located chiefly along the flood plains of the principal irrigation streams. It is probable that some of these may have originated from the lodgment of infected driftwood on the bottomlands during the flood season or in the distribution of irrigation water.

In some of the irrigation projects the lands nearest the rivers and those with the oldest riparian rights show more extensive infestations than the lands to which the water was only recently applied. Infestations have been found in the watershed areas of some of the larger projects, and in one case infected native plants and spore mats were found in several places on the steep hills surrounding the principal irrigation reservoir and many miles upstream from the nearest irrigated fields.

In 1933 the writers observed an extraordinary behavior of root rot in some cotton plantings on newly cleared land near Coolidge, Arizona. The area, before clearing, was of the mesa type made up of a highly calcareous soil with outcroppings of "caliche," and was covered largely with the creosote bush *Covillea tridentata*. The area was traversed by 3 or 4 washes that drained northwestward toward the Gila River. In leveling the land for crops these washes were filled in places where they were shallow, but were largely left open and cotton was planted as close to their banks as possible. In Oct., 1933, it was noted that areas of dead cotton plants lay in long narrow strips along the banks of the washes. In some cases these irregular strips were continuous for more than $\frac{1}{4}$ mile (Fig. 1), and the tendency of paralleling the stream beds was noted for nearly $1\frac{1}{2}$ miles.

Root-rot infestations can be found to some extent in nearly all of the tilled areas along the Gila River throughout the 350 miles of its course, from western New Mexico to its junction with the Colorado River at Yuma, Arizona. Infestations along the Colorado River system are known as far north as Washington County, Utah, and are extensive in some of the irrigated areas near Blythe, Calif., and Parker and Yuma, Arizona.

In reporting the discovery of the infestation in southern Utah (Plant Disease Reporter, May 15, 1933) B. L. Richards of the Utah Agricultural College stated as follows:

"A survey in Washington County, Utah, discovered for us the presence of *Phymatotrichum* root-rot in this particular locality. At present the disease is spread generally throughout the townships of Ivins, Santa Clara, St. George, and Hurricane, and is doing tremendous damage to the various crops. It is evident from our survey that the organism has been in the county probably since settlement by the pioneers and is probably indigenous to the region, as a number of fields exhibited root-rot disease the first year after being reclaimed from the native vegetation."

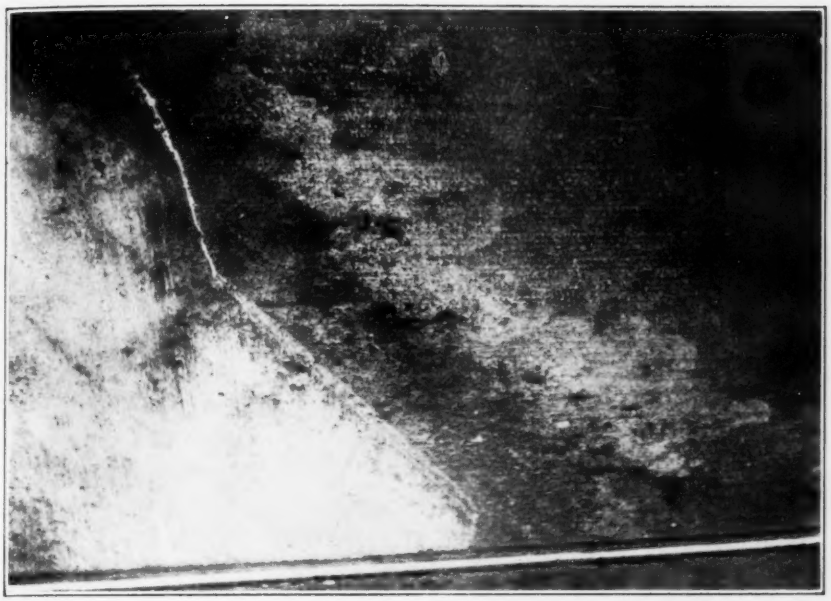

FIG. 1. From an aerial photograph showing irregular streaks of cotton plants killed by root rot lying parallel to a desert wash. For many years before the land was cleared of native vegetation the wash had served as a waste channel for river flood water escaping from irrigated fields some of which were infested with the root-rot fungus. The dark area nearest the channel represents nonsusceptible weeds, mostly *Portulaca oleraceae*. The irregular high streaks represent the dead areas of cotton plants that, in the photograph, are continuous for about ¼ mile. They are bordered by healthy areas at the extreme right and left.

Further explorations in the watershed areas of some of the stream beds of the Southwest may disclose the fact that root rot is not uncommon at the higher elevations. Transmission of infected roots and of sclerotia from these higher areas by erosion and drainage affords a possible explanation of some of the infestations in the cultivated areas of lowlands and deltas.—C. J. KING, CLAUDE HOPE, and E. D. EATON, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Control of Apple Sooty Blotch by May and June Sprays.—Sooty blotch of apples is not generally considered a very destructive disease, but in some seasons it produces a serious blemish on apple fruits. The fact that the disease is not evident until late in the season (August) has led to the opinion that late-summer sprays are necessary to its control. However, in spraying tests conducted at La Fayette, Indiana, in 1928 and 1932, it was controlled when the last fungicidal spray was applied in early June.

This period corresponded to the time of maturity and dissemination of the spores of the fungus.

The data[1] reported here were collected incident to spray tests for the control of apple scab and codling moth conducted by the Department of Horticulture of the Purdue University Agriculture Experiment Station. The experimental orchard consisted of 175 19-year-old, normally productive apple trees. A row of persimmon trees and an open woods bordered the east side of the orchard. Branches of the persimmon trees were heavily infected with sooty blotch and possibly were the chief sources of inoculum.[2]

In 1928, the experimental orchard was divided into 2 plots, which contained approximately equal numbers of Jonathan, Stayman, Grimes, and Rome Beauty trees. One plot was sprayed, receiving a pink, calyx, and first-cover spray of liquid lime sulphur and lead arsenate, and a second and third cover spray of lead arsenate and lime. The liquid applications averaged 5½ gallons per tree. The other plot received 12 applications of an 85–15 dust (85 per cent colloidal sulphur and 15 per cent lead arsenate), of which 7 were applied to both sides of the trees. The dust was applied at night at the application rate of 1 lb. per tree. The last fungicidal spray was applied on June 9 and the last dust on August 11, with 4 dust applications in June. One Rome Beauty and 1 Jonathan tree were left without treatment.

At the end of the season apples were counted from 2 Rome Beauty, 2 Stayman, 1 Grimes, and 1 Jonathan tree in the sprayed plot and from 2 Rome Beauty, 1 Stayman, 2 Grimes, and 1 Jonathan in the dusted plot and from the Rome Beauty and Jonathan checks. The fruit from the 4 varieties in the sprayed plot averaged 87.3 per cent clean, while the dusted plot averaged 67.3 per cent. The blotched fruits from the sprayed plot were less severely infected than those from the dusted plot. Only 8.5 per cent of the fruit from the Jonathan check and 2.7 per cent from the Rome Beauty check were clean and many of the infected fruits were nearly blackened by the fungus coating.

In 1932, 2 Stayman trees sprayed with Flotation sulphur, 3 Staymans sprayed with Ansul sulphur, and 1 Rome Beauty sprayed with Bordeaux mixture were selected for sampling from the plots that received applications of the respective sprays at the pink, petal-fall, and 3 and 5 weeks after petal-fall stages. Lead arsenate was used throughout as the insecticide, except for the first and second cover sprays in the Flotation-sulphur plot,

[1] Data for 1928 from: Zaring, Doyle, "Studies on sooty blotch of apple." Thesis for degree of M.Sc. in Agriculture, Purdue University, 1929. Copies on file in Library and in Department of Horticulture of Purdue University.

[2] Recent tests by the writer have shown that the disease can be produced on excised green apples in a moist chamber by atomizing with a spore suspension from a culture of the sooty-blotch fungus isolated from persimmon.

where Dutox and Verdol were substituted. A Rome Beauty and a Stayman tree, sprayed with liquid lime sulphur 1–50 in the pink and petal-fall stages only, served as checks on the 2 later applications of fungicides.

From the trees sprayed with Bordeaux mixture, Flotation sulphur, and Ansul sulphur, 99.5, 97.9, and 94.1 per cent of clean fruit, respectively, were obtained, while 37.5 and 57 per cent of clean fruit were secured from the respective Rome Beauty and Jonathan check trees.

The 1928 and 1932 results indicate that effective control of sooty blotch can be obtained if fungicides are applied just prior to the limited period of spore dissemination. The last fungicidal sprays were applied on June 9, 1928, and June 13, 1932.

Baines and Gardner[3] observed that spore liberation occurred during a rain on June 5 and 6, 1931. On May 31, 1932, many immature and few mature spores were observed on the stems or branches of 8 of the following 10 hosts: leatherwood, smilax, willow, false bitter sweet, prickly ash, sassafras, sumac, bladder nut, maple, and hawthorn. On June 11 an abundance of mature spores were observed on all of the above plants. Microscope observations indicated that heavy spore dissemination from all of these hosts apparently was limited to the rainy period of June 15 to 18, 1932.—RICHARD C. BAINES,[4] Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Indiana.

Elimination of Potato Late Blight from North America.—Species of tuberous solani exist in the highlands of central Mexico that are immune from the late blight or mildew caused by *Phytophthora infestans* (Mont.) de Bary. The following species are known to possess this immunity: *Solanum demissum* Lindley, *S. coyoacanum* Bukasov, *S. neoantipoviczii* Bukasov, *S. antipoviczii* Bukasov, *S. verrucosum* Schlecht. (in part), *S. polyadenium* Greenm., *S. sambucinum* Rydb., *S. bulbocastanum* Dunal, *S. ajuscoense* Bukasov. None of the species is of commercial importance. The plants are not large, the stolons are long (up to 2 m.), the tubers vary in size from 1 to 5 cm., and none of the species begins tuber production until the length of day is 12 hours or less. Furthermore, cross-fertilization between some of these species *inter se* or with varieties of the cultivated potato has not yet been accomplished, although all of the species are self-fertile. Curiously enough, *S. demissum* with 72 chromosomes (diploid) has given the most hybrids with cultivated sorts (48 chromosomes).

[3] Baines, R. C., and M. W. Gardner. Pathogenicity and cultural characters of the apple sooty-blotch fungus. Phytopath. 22: 937–952. 1932.

[4] The writer wishes to express his appreciation to C. L. Burkholder of the Department of Horticulture of Purdue University, for his cooperation in securing the data presented here.

Hybrids in which *S. demissum* is the male parent, however, are rare because fertilization is not accomplished. On the other hand, a generous quantity of seed can be obtained with *S. demissum* as the female parent. The difficulty here is to find domesticated sorts that produce good pollen and, particularly, commercial varieties that possess this ability. The new variety Katahdin and some of its sibs and some unnamed varieties developed by F. A. Krantz in Minnesota really have made possible a favorable outcome of an endeavor that, at the outset did not offer much encouragement.

At Ithaca, thus far, *S. demissum* as pollen parent has given a very scant set of seeds only 5 times and each time on variety Rural or on Rural hybrids. The first-generation hybrids are intermediates in many characters, are all blight-immune and are practically male-sterile. Less than 100 individuals of the second generation have been produced. A segregation occurs and blight immunity is found to be heritable, although it is not possible to determine the probable mode from such meager material. Also, some of the progeny have the general appearance of the Rural parent with fairly short stolons and tuber production before autumnal equinox.

When *Solanum demissum* is the female parent and fertile pollen from cultivated varieties is employed, the first-generation hybrids usually resemble the wild parent rather closely, although some intermediate characters may be observed. Practically all of the plants of this generation are immune from blight, (90 per cent at present, but some of the *demissum*-like parents are of questionable genetic purity), they develop long to very long stolons, and a good many of them persist in the habit of producing tubers only with short day. Such hybrids are mostly fertile. In the second and succeeding generations (at least as far as the fourth) all plants tested have reverted to the wild type; all are immune from blight and continue so in succeeding generations.

If an immune first-generation plant is backcrossed with pollen from a cultivated variety the progeny is heterogeneous. Subject to the reservations noted above, 68 per cent (1762 plants tested) of the backcross plants are immune from blight, most of them are much larger than *demissum* and some are larger than either parent, the stolons tend to be decidedly shorter than in the first generation, and a fair proportion of the plants produce tubers approximating commercial size and before the equinox. As the plants grow in the field their *demissum* parentage is noticeable especially in foliage and flower.

It is not known yet what is to be expected from a selfed backcross, but when a blight-immune backcross plant is again backcrossed with pollen from a cultivated variety the progeny is again heterogeneous, this time inclining in general aspect to the cultivated type. Records exist for 767 such double backcross plants and of these 50 per cent are immune from

blight. About 200 blight-immune double-backcross plants grown from seeds started on May 15, 1933, were set in the field on June 30 and subjected to repeated natural infection in late September and early October. No infection occurred, nor was it expected, because of the severe elimination tests in the greenhouse in mid-June. From this lot a dozen plants were selected that had the appearance of domestic varieties and which bore from 6 to 9 tubers of commercial size, smooth and white, with fleet eyes, and on short stolons. About a dozen other selections were made of plants approximating commercial types and size and from previous trials with seedling plants it may be confidently predicted that several of them will yield tubers of larger size in 1934.

Many of the double-backcross plants set seed freely when selfed, so that it will be known soon what such selfings will yield; and also whether these plants can be used to transmit blight immunity to the well established commercial varieties practically all of which are male-sterile.

While it is felt that the title of this note is justified as a not too distant possibility, it is recognized fully that the development of these new blight-immune varieties may serve merely to demonstrate the existence of clear-cut biologic specialization of *Phytophthora infestans* on the potato in North America, just as similar work in Germany has brought out the existence of biotypes in that country. A summary of the situation in North America has been published recently in another place.[1] The collaboration of interested persons is sought not only to determine the integrity of the blight immunity of these varieties throughout the late-blight area but also to determine the adaptability of the varieties for commercial production. Small samples for experimental purposes should be available in 1935, assuming that a disease that has become prevalent recently at Ithaca does not ruin everything. This disease is apparently the one described by Barrus[2] under the name "streak." It had not been observed again at Ithaca, except perhaps sporadically, until the summer of 1932. Every effort was made to eliminate the disease by tuber indexing and roguing in 1933 but without success. Such incidents merely emphasize the importance of disseminating widely any likely new varieties at the earliest possible time.—DONALD REDDICK, Cornell University, Ithaca, N. Y. (Cooperative work with the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture).

[1] Reddick, Donald, and Willard Crosier. Biological specialization in *Phytophthora infestans*. Amer. Potato Jour. **10**: 129–134. 1933.

[2] Barrus, M. F. Physiological diseases of potatoes. Ann. Rept. Quebec Soc. Prot. Plants, **9**: 45–53. 1917. Streak is described on p. 51 and the 3 illustrations refer to this disease.

Verticillium Wilt of Cotton in Greece.—The wilt caused by a species of Verticillium, commonly designated as *Verticillium albo-atrum* R. & B., was first recorded as occurring on cotton under field conditions by Sherbakoff[1] in Tennessee, in 1928. The following year he recorded its discovery on the opposite side of the Mississippi River in Arkansas.[2] The disease was noted in 1931 by Rudolph[3] on cotton from California and from Mississippi by Miles and Persons[4] in 1932.

Recent correspondence between the author and Dr. J. A. Sarejanni, Plant Pathologist at the Institut Phytopathologique Benaki, Kiphissia-Athenes, Greece, has indicated that the trouble has recently made its appearance in that country. The following is a translated excerpt from a letter from him dated February 22, 1933:

"In the month of September of last year I was called to the region of Copias to diagnose a new disease of cotton. I was able to establish that it was a question of wilt or hadromycosis. I was, however, greatly astonished to see appear from twenty cultures nineteen of pure Verticillium and a single one of Fusarium. Happily, however, your recent article with Mr. Persons,[4] which arrived soon after, cleared me up on the matter.

"These two hadromycoses or wilts, that of the Verticillium as well as the Fusarium, were observed at this time for the first time in Greece. I question if they have not come from America, since I have never found them except in fields planted with seed imported from Carolina in the United States in 1932. Is it possible that Verticillium wilt of cotton is perhaps more widespread in the United States than has been believed?"

In replying to this letter a request was made that cultures of the Verticillium isolated from cotton in Greece be sent for comparison with those isolated from cotton in Mississippi. These cultures have recently been received and are identical in growth characters of the colonies and in morphological details of the fungus with those secured from cotton in Mississippi and also with those cultured from cotton in Tennessee by Sherbakoff in 1928.

The following excerpt from a letter from Sarejanni, dated January 24, 1934, shows the present status of the disease in Greece:

"The hadromycosis (wilt) due to Verticillium has spread throughout the whole of Greece in the course of this year. I have isolated it in the north in Macedonia as well as in the south in continental Greece. From all the isolations which I have made this year from various specimens drawn

[1] Sherbakoff, C. D. Wilt caused by *Verticillium albo-atrum*. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Sup. 61: 283–284. 1928. (Mimeographed.)

[2] Sherbakoff, C. D. Verticillium wilt of cotton. Tenn. Agr. Exp. Sta. Circ. 24: 2. 1929.

[3] Rudolph, B. A. Verticillium hadromycosis. Hilgardia **5**: 197–354. 1931.

[4] Miles, L. E., and T. D. Persons. Verticillium wilt of cotton in Mississippi. Phytopath. **22**: 767–773. 1932.

from different localities I have always secured Verticillium and never *Fusarium vasinfectum,* which latter I have isolated but a single time last year from Livadia.

"From the simultaneous appearance of the trouble in several provinces quite distant from each other, as well as from the fact that the appearance has followed the introduction of foreign varieties, I am much inclined to persist in the hypothesis of seed transport. In spite of experiments made in this direction, however, I have not been able to demonstrate the fungus on the interior of seeds borne on diseased plants. . . . I do not believe definitely that the disease has not existed in Greece previous to that date (date of introduction of foreign seed) simply for the reason that it had not been pointed out to me, but I should feel secure in saying that the introduction of American seed has brought about heavy infections in the cotton sections.

"The Cotton Institute recently sent me the following information regarding cotton seed which it introduced from America in 1932.

"Pedigreed Cleveland, 5 tons from North Carolina.

Acala, 20 tons
Ingold, 10 tons
Trice, 15 tons
King, 45 tons

These four varieties were furnished by T. W. Wood & Sons, Richmond, Virginia. The point of origin of these seeds was not specified."

Experiments of the author and of other workers in this country are in agreement with those of Sarejanni in an inability to demonstrate the occurrence of the organism within the cotton seed. These experiments, however, have not been extensive enough to warrant a conclusion that the disease may not, at least sometimes, be transmitted in that manner. There exists the hypothesis, suggested by Sarejanni in other correspondence, that the organism occurs, not within the seed, but on the seed held by the fuzz or lint with which it is covered. There is also the possibility that the organism was already present in the soils of Greece, possibly attacking, or present without evident damage on, some other host plants, and that the cotton commonly grown in Greece is immune from or resistant to its attack. The varieties of cotton imported from the United States may have been readily susceptible to the attacks of the Grecian strain already present, thereby presenting what would seem to be evidence in support of the assumption that the causative organism had been introduced on such imported seed. L. E. MILES, Mississippi Agricultural Experiment Station, State College, Mississippi.

Variation in Hyphal-tip Cultures from Conidia of Helminthosporium gramineum.[1]—Hyphal-tip cultures isolated from individual germinating conidia were used in a study of variation in *Helminthosporium gramineum* Rabh. Each isolant, containing at least one cell with a septum, was severed from the advancing germ tube. Colonies grown from hyphal tips, isolated

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

directly from conidia produced on striped barley leaves, minimized the artificial culture effects on the fungus. This also made possible the observation of some of the variations that most probably existed in the conidium at the time of germination. A series of isolations involving from 2 to 5 hyphal tips from each of 5 conidia was made in October, 1932, and a second series, involving hyphal tips of 20 additional spores, was made in August, 1933. The hyphal-tip cultures have been studied on media and in inoculation trials.

Hyphal-tip cultures from the same conidium reacted differently in Petri dish culture on potato-dextrose agar, in pathogenicity tests, and in symptoms produced on the barley plants. The type of mycelial growth and pigmentation varied widely in hyphal-tip cultures from a conidium. These cultures from the same spore were found to vary from almost nonpathogenic to highly pathogenic when seed inoculations were made on Wisconsin Pedigree 5–1 barley, grown in 1932. The cultures from some spores showed less pathogenicity range than did others. The disease symptoms produced by cultures from certain conidia showed further variations. The striped plants produced by inoculations with some hyphal-tip cultures developed characteristic leaf lesions on plants that were essentially the same height as the controls. The diseased plants from inoculations with other cultures from the same spore were mostly dwarfed and rosetted. The infected plants from cultures of other spores developed both types of striped plants. Thus it is apparent that a conidium may carry within its cytoplasmic and nuclear composition more than one cultural type, and more than one pathotype. There is evidence that some cultures are more stable than others, as repetition of pathogenicity trials has shown some cultures to be more consistent in producing the same percentage of stripe and type of symptoms. However, a hyphal-tip culture of *Helminthosporium gramineum* seemingly does not necessarily guarantee purity and stability of characters. Limited monohyphal reisolations in one of the more stable cultures have indicated that the new isolants resembled the parent rather closely, which suggests that a condition of stability in cultural characters, pathogenicity, and disease symptoms is being approached.

Cytological preparations in which Flemming's fixative and Heidenhain's Haematoxylin stain were used have shown germinating conidia, germ tubes, and mycelial cells to be multinucleate. With the multinucleate condition prevalent in the conidium, heterocaryosis may possibly exist. This might serve as a basis for explaining variations found in cultures obtained by the isolation of germ-tube hyphae from the same conidium. The results of pathogenicity, cultural, physiological, and cytological investigations of hyphal-tip cultures will be reported more completely at a later date.—H. L. SHANDS and JAMES G. DICKSON, Plant Pathology Department, University of Wisconsin, Madison, Wisconsin.

BOOK REVIEW

The Efficacy and Economic Effects of Plant Quarantines in California. By H. S. Smith, E. O. Essig, H. S. Fawcett, G. M. Peterson, H. J. Quayle, R. E. Smith, and H. R. Tolley. University of California Bulletin 553: 276 pp. 1933.

The American reading public—even the scientific public—still shows great respect for board covers and cloth bindings, and particularly for those volumes that are sold by great commercial publishing houses. It is possible then that the book under review, which comes to us as Bulletin 553 of the California Experiment Station, may not receive the general attention it richly deserves.

Two economists, 2 plant pathologists, and 3 entomologists, each of recognized standing in his own field, collaborating over a period of 3 years, have produced a volume that contains a discussion of the social-economic aspects of changes in crop volume, whether caused by disease or otherwise. This discussion is clearly stated in nontechnical language and can be read easily and profitably by anyone really interested in these questions. It nevertheless deserves the thoughtful consideration of those professionally interested in the control of plant pests and diseases.

Since the seven authors form a committee appointed for the specific purpose of studying the "Efficacy of plant quarantines as applied to California and their economic effects" there, it is inevitable that much of the matter will be of interest only to limited groups. The section on "Administrative aspects of plant quarantine," pages 82–99, will be read chiefly by those professionally interested in quarantine problems. This is true in even greater degree of the section that deals with the history and administration of plant quarantines in California, pages 104–139. Most of the analyses of the specific quarantines now in force in California, 38 in number, will probably be read carefully only by students of the particular insects or diseases involved.

It is in the introductory sections, particularly in the sections on economic aspects, that the most stimulating material is found. In his foreword, Dean Hutchison, who appointed the committee, advances the belief that, in view of the national and international relations, it would have been desirable to have had the study undertaken by some national agency, preferably one with no connection with either Federal or State governments. He says also that he sought and failed to get such assistance. The committee, however, has included much matter of general significance. Following the foreword, there is a discussion of the biological aspects of plant quarantine in which is included a general review of the distribution of

plant pests and diseases and the methods of their dispersal, the probability of introduction, of establishment, and of their becoming important. This matter, while well stated and appropriately included, is in large part well known and generally admitted.

When, on page 42, the committee attacks the question of the effect of pests on the total social income and on the distribution of income among individuals and groups they enter ground too often avoided or slighted, but of fundamental importance not only to biologists but to the general public.

Specific illustrations are given of the *immediate* effects on growers of changes in volume of crop (p. 51) and its complexity summarized in the statement:

"Sometimes the growers as a group or class might gain, in other cases they would lose—a small change in volume may have one tendency, a large change just the opposite."

Particularly refreshing in view of the recent tendency in some quarters to regard disease control as of concern only to the producers, are the conclusions of the committee when it comes to consider the broader aspects of losses from diseases or pests, which may be best stated in quotation:

"Society considered as a large group of producers and consumers has interests almost identical with those of individual producers."

"In the long run the consumers tend to pay all of the costs and to receive all of the benefits [of pest and disease control measures] except when some form of monopolistic control intervenes and then consumers continue to pay all of the costs but do not receive all of the benefits."

"*The consumer always benefits from an abundance of production and should be more interested in maintaining plant quarantines that insure such abundance than growers as a group.*"

The italics are in the original, though I should be glad to accept the responsibility for their use. And, if it were permissible in a review, I should move to amend by striking out "Maintaining plant quarantines" and inserting "All plant-pest and disease-control measures."

On the validity of the committee's conclusions as stated in the last ten pages, I do not feel competent to pass. As would be expected of skilled and experienced investigators, the authors fully realize that they are not saying the last word. Indeed, to quote again from Dean Hutchison's foreword—"The committee feels that it has made only a beginning." May I be permitted to add that it is a good beginning.—NEIL E. STEVENS, Bureau of Plant Industry, Washington, D. C.

REPORT OF THE TWENTY-FIFTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE BOSTON MEETING

The twenty-fifth annual meeting of The American Phytopathological Society was held from December 28 to 30, 1933. Approximately 150 members were present and 67 papers were presented at 7 sessions. There were joint sessions with Section G, with the Mycological Society of America, and the Potato Association of America.

The contributions presented at this meeting may be classified according to subject as follows: Taxonomy, 11 papers; virus diseases, 9 papers; diseases of vegetables, tobacco, cotton, and other crops, 13 papers; diseases of fruits, 9 papers; diseases of cereals and forage crops, 8 papers; diseases of ornamental and forest trees, 9 papers; and potato diseases, 8 papers.

The annual conference on extension work in plant pathology, held December 28 under the chairmanship of Charles Chupp, was devoted to a discussion of the following subjects: (1) New seed disinfectants and fungicides, (2) commercial seed treatment, its advantages and limitations, (3) seed treatment and other service work in the counties, (4) emergency-garden relief work, (5) new points on illustrative material and its use.

A session on pathological activities in emergency and recovery programs, under the chairmanship of A. F. Woods, brought to light the important part that plant pathologists are at present taking in the national recovery programs and called attention to opportunities for plant pathologists to render service under present economic conditions.

On December 28 was held the annual dinner, entertainment being supplied by Wm. H. Weston, Jr. (Harvard University), W. L. Doran (Massachusetts State College of Agriculture), F. A. Wolf (Duke University), and Fred Sparrow (Dartmouth College).

Abstracts of the papers on the program of this meeting are printed in the January number of PHYTOPATHOLOGY.

OFFICERS AND REPRESENTATIVES

The following officers and representatives were chosen:

President, N. E. Stevens, U. S. Department of Agriculture, Washington, D. C.

Vice-President, G. W. Keitt, University of Wisconsin, Madison, Wis.

Councilor (two years), F. D. Heald, State College of Washington, Pullman, Wash.

Associate Editors (three years), Glenn G. Hahn, Yale University, New Haven, Conn.; R. P. White, State College of Agriculture and Mechanic Arts, New Brunswick, N. J.; F. O. Holmes, The Rockefeller Institute for Medical Research, Princeton, N. J.; and J. J. Christensen, University Farm, St. Paul, Minn.

Business Manager (one year), F. C. Meier, U. S. Department of Agriculture, Washington, D. C.

Advertising Manager (one year), R. S. Kirby, Pennsylvania State College, State College, Pa.

Representatives on the Council of the A. A. A. S. (one year), N. E. Stevens, U. S. Department of Agriculture, Washington, D. C., and H. S. Cunningham, Long Island Vegetable Research Farm, Riverhead, N. Y.

Member of the Board of Governors Crop Protection Institute (3 years), J. F. Adams, University of Delaware, Newark, Del.

Elector to Division of Biology and Agriculture of the National Research Council, John W. Roberts, U. S. Dept. Agr., Washington, D. C.

The following temporary committees were appointed to serve throughout the meetings:

Auditing Committee, John W. Roberts and John Stevenson.
Committee on Elections, A. N. Brooks and H. S. Cunningham.
Committee on Resolutions, W. H. Tisdale, Annie R. Gravatt, and S. M. Zeller.

REPORT OF THE SECRETARY-TREASURER, 1933

The Society started the year 1933 with 827 members. During the year 5 former members were reinstated, but we lost 84, of which number 21 resigned, 3 died, and 60 were dropped from the Society for nonpayment of dues. Each of the 60 suspended received 3 statements and a letter but there was no response to any of our communications. Thus, at the beginning of the winter meetings, our membership stood at 748. Election of the 26 new applicants for membership brought our membership to 774 for the beginning of 1934. Of this number 100 are paid up life members and 70 are life-sustaining.

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING NOVEMBER 30, 1933

Receipts:

Balance from 1932			$1,849.18
Annual Dues:			
1930	$ 5.00		
1931	5.00		
1932	34.40	($ 10.00 life)	
1933	1,661.02	(259.85 life)	
1934	1,358.69	(380.00 life)	
1935	22.05	(10.00 life)	3,086.16
Interest on checking account			9.53
Donations to Lyman Fund included in checks for dues			7.86
Sales and special sales in checks for dues			5.95
Classics included in check for dues			0.75
Checks to replace those returned by bank due to bank holiday, etc.			65.00
Refund by Sec.-Treas., expenses of Atlantic City meeting			3.88
Cash from B. B. Mundkur for dues in other societies			7.15
Share of A. P. S. in distribution of surplus funds of International Congress of Plant Sciences			179.58
Total Receipts			$5,215.04

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY (1933, part 1932)	$2,532.52
Transferred to Sinking Fund savings account	395.00
Secretarial work	177.56
Printing (preliminary programs, ballots, etc.)	66.53
Postage and stamped envelopes	75.70
Telephone and telegraph	17.33
Advance of funds to Secretary-Treasurer for Atlantic City meeting	50.00
Expenses of Centennial Exhibits Committee	11.00
Checks returned by bank and discount on check	81.70

Donations transferred to Lyman Fund	7 86
Sales and special sales transferred to PHYTOPATHOLOGY	5 95
Classics transferred to H. H. Whetzel	0.75
Dues in other societies, B. B. Mundkur	7.00
Supplies	1.00
Federal tax on checks	0.64
Share of cost of electrical transcription	2.50
Balance on hand	1,782.00
Total Expenditures	$5,215.04

SINKING FUND

The Sinking Fund, obtained by deducting $5.00 (formerly $6.00) from each life-sustaining membership payment, now stands at $7,986.00, of which $5,000 is invested in first mortgage notes. $2,781.00 is deposited in a savings account with the Riggs National Bank of Washington, D. C. The remainder, $205.00, has not yet been transferred from the checking account of the Society. Income from this fund now amounts to almost $375.00 and is used to help defray the cost of publishing PHYTOPATHOLOGY.

Respectfully submitted,

F. C. MEIER, *Secretary-Treasurer.*

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1933

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING NOVEMBER 30, 1933

Receipts:

Balance from 1932		$1,183.79
Subscriptions:		
1931	$ 1.24	
1932	24.83	
1933	2,311.36	
1934	305.61	2,643.04
Member subscriptions, 1932 and earlier		32.52
Member subscriptions, 1933		2,500.00
Sales of back volumes and numbers		106.05
Special half-price sale		208.15
Advertising:		
1932	$ 87.16	
1933	417.20	504.36
Interest on Sinking Fund		342.45
Checks to replace those returned by bank during bank holiday, etc.		50.37
Dues in The American Phytopathological Society included in checks with subscriptions		15.00
Dominico Pirone for Phytopathological Classic No. 2		200.00
Association of Hawaiian Pineapple Canners for share of cost of Carter articles		125.00
Rockefeller Institute for cost of Price article		106.27
Total Receipts		$8,017.00

Expenditures:

Printing and distributing PHYTOPATHOLOGY:			
Vol. XXII, No. 12	$378.31		
Index to Vol. XXII	182.50	$ 560.81	
Vol. XXIII, No. 1	$621.62		
No. 2	540.98		
No. 3	630.20		
No. 4	541.53		
No. 5	549.95		
No. 6	366.14		
No. 7	343.80		
No. 8	338.78		
No. 9	374.15		
No. 10	573.04	4,880.19	
Postage		447.29	$5,888.29
Secretarial work for Business Manager			176.06
Expenses of Office of Editor in Chief			292.73
Commission of former Advertising Manager			40.91
Part commission of present Advertising Manager			42.00
Expenses of Advertising Manager			26.80
Stamps and envelopes			102.54
Printing			46.98
Transferred to The American Phytopathological Society for dues			15.00
Checks returned by bank and exchange charge			68.30
Telephone and telegraph			4.26
Subscription refunded			5.53
Printing Classic No. 2			186.84
Incidental expenses, handling Classic No. 2			17.27
Binding PHYTOPATHOLOGY for official set			34.65
Federal tax on checks			0.80
Supplies			0.40
Balance on hand			1,067.64
Total Expenditures			$8,017.00

At the end of the year 1932 there were 536 subscribers (nonmembers) to PHYTOPATHOLOGY, including 7 complimentary subscriptions. Fifty-one (of which 27 were from U. S. S. R.) were cancelled for 1933 and during the year 32 were dropped for nonpayment of subscriptions. With the addition of 49 new subscribers, the net loss was 34, reducing the list to 502.

Note: In order to facilitate preparation of the annual report at a time when his work in connection with the Society is always heavy, the Secretary-Treasurer of The American Phytopathological Society, who is also Business Manager of PHYTOPATHOLOGY, closed the accounts for both Society and Journal at the end of November this year. This makes the apparent receipts and expenditures considerably less than would normally be the case for a full year, as more business is transacted during the first 2 weeks of December than in any other similar period of the year.

Respectfully submitted,

F. C. MEIER, *Business Manager.*

REPORT OF THE COMMITTEE ON LYMAN MEMORIAL FUND

The Lyman Memorial Fund has grown during the period, January 1, 1933, to December 31, 1933, from $1,679.77 to $1,870.47. This includes contributions of $138.86 and interest amounting to $51.84. The Fund has, therefore, grown by about $200.00 during the past year.

One of the principal features was the fact that the members of the Plant Pathology Department, of the New Jersey Experiment Station, contributed $63.00. The Committee wishes to make public acknowledgment and thank the members of the Department for their generosity.

No particular drive was made for contributions, because of the economic conditions. Neither were any munificent bequests obtained. Nevertheless, the Fund has grown gradually and steadily, even though rather slowly, and the Committee feels that efforts should be continued and intensified as soon as improvement in economic conditions warrants.

Respectfully submitted,

E. C. STAKMAN, *Chairman.*

REPORT OF THE EDITOR IN CHIEF

In my report submitted a year ago, at Atlantic City, reference was made to the effect of the "depression" on the printing budgets of plant-science journals and the general absence of any diminution in the tide of manuscripts submitted for publication in PHYTOPATHOLOGY. Again, even though, as some assert, we have not yet sighted the cloud of silver lining, the volume of manuscripts submitted to your editorial board has shown no sign of depression. Indeed, during the 6-year period, 1928 to 1933, the rate of arrival of manuscripts on the editor's desk has increased from one manuscript every 4 days to one every 2½ days.

Somewhat contrary to expectation, this increase in volume has been marked by a general improvement in the scientific and literary merit of the several manuscripts. During the past year, particularly, the improved excellence of papers contributed has been notable and encouraging. The members of the editorial board have, however, found it necessary to recommend drastic revision of a number of manuscripts, and compliance with their recommendations has, with very few exceptions, been cheerfully accorded. Whatever may have been their just deserts, your editors have received among several epistolary bouquets very few of the anticipated prickly-pear variety. It would prove salutary to the editorial staff if your modesty would give way occasionally to some constructive criticism, for PHYTOPATHOLOGY is *our* journal and the editors desire above everything else to make it fully worthy of the scholarship and the best scientific effort of our membership.

The recommendation proposed and adopted by the Society in business session at the Atlantic City meeting, calling for the preparation of author abstracts of manuscripts submitted for publication in PHYTOPATHOLOGY, has been in practical operation for the past several months. Abstracts thus far submitted have been forwarded to BIOLOGICAL ABSTRACTS for publication in that journal.

The 23rd volume of PHYTOPATHOLOGY contains 1006 pages of printed text and illustrations, or 4 pages more than comprised Volume 22. The 1006 pages of our journal for 1933 are classified as follows: 78 articles, 36 phytopathological notes, 2 reports, 7 book reviews, 124 abstracts (2 by title only), and 212 text figures. During the period January 1 to December 31, 1933, inclusive, approximately 153 manuscripts of articles, phytopathological notes, reports, book reviews, etc., and 64 abstracts (1 by title only) were submitted. Of this number 12 were returned to their authors for revision, 12 were found unacceptable, and 5 were withdrawn by their authors. Of the manuscripts re-

ceived in 1932, too late for publication that year, 2 were returned, in the current year, for revision. In addition to the several papers published this year, 9 articles, 11 phytopathological notes, 1 book review, and 57 abstracts (1 by title only) are now (December 31) in press. The index for Volume 22 was published as a supplement to the February, 1933, number of PHYTOPATHOLOGY.

The editor again wishes to express his appreciation and gratitude in behalf of the excellent clerical and editorial services rendered by Frances W. Todd. The thanks of the editor are here extended to the several members of the editorial board who have given so much of their time and effort to duties incident to the publication of our journal and to The Science Press Printing Company for its high-grade workmanship in printing PHYTOPATHOLOGY.

Respectfully submitted,

H. B. HUMPHREY, *Editor in Chief.*

REPORT OF THE BUSINESS MANAGER, PHYTOPATHOLOGICAL CLASSICS

I beg to submit herewith the annual financial report covering the receipts and expenditures in connection with my stewardship as manager of Phytopathological Classics for the year 1932–33.

Total number of Classics No. 1 *received*	647
Total number of Classics No. 1 *sold*	196
Balance on hand	451
Total number of Classics No. 2 *received*	1012
Total number of Classics No. 2 *sold*	330
Total number of Complimentary copies	26
Balance on hand	656
Total number of Classics No. 3 *sold*	2

The above classics were sold in the following manner:

Classic No. 1 — 19 copies at $ .50	$ 9.50	
Classic No. 2 —153 " " .50	76.50	
Classic No. 1 and 2—174 " " .75	130.50	
Classic No. 1 and 2— 1 copy " .74	.74	
Classic No. 1, 2, & 3— 2 copies " 1.25	2.50	
Total receipts from sale of classics		$219.74
Balance from Pirone check		$ 13.16
Total receipts		$232.90

Expenditures itemized:

Express charges	$ 1.69	
Envelopes and letterheads	16.00	
Advertising, reprints	5.58	
Postage	21.38	
Total expenditures		44.65
Balance of money on hand		$188.25

In December of last year I received from the Secretary of The American Phytopathological Society the residue copies of Classic No. 1, a total of 647. I also received from his hands the total number of Classic No. 2, 1012 copies. Classic No. 3, "The Discovery of Bordeaux Mixture," 3 papers by Millardet, translated by F. J. Schneiderhan, are now in press and will be ready for distribution at the Boston Meeting at 50 cents each. The above report is of December 8. You will note that on this date 2 advanced sales of Phytopathological Classic No. 3 were made, but as Classic No. 3 was not then off the press the cost of same does not appear in the items of expenditure, for the past year, but will appear next year.

Although the total of Classics No. 1 and No. 2 sold during the past year is not extraordinarily large, being 196 for Classic No. 1 and 330 for Classic No. 2, still, the net income from these sales including 2 copies of Classic No. 3, totaled $188.25. This sum is much more than sufficient to pay for the publication of 1000 copies of Classic No. 3. I should recall to you that the cost of publication of Classic No. 2 was donated by Mr. Dominico Pirone, father of Dr. P. P. Pirone, the translator. In fact, there was an item of $13.16 cash balance from the check sent me by Mr. Pirone after the cost of publication of Classic No. 2 had been met. This $13.16 is also included in the net $188.25.

You will be interested to know that manuscript for Classic No. 4 is nearly ready for publication. It is a translation of Woronin's paper on Club Root of Cabbage. This has been translated by Dr. Charles Chupp, of Cornell University. The publication of the translation will include, of course, the plates illustrating Woronin's original paper. It is expected that the income from the sales of Classic No. 3, with additional sales of Classics No. 1 and No. 2, will provide a sum sufficient to pay for publication of Classic No. 4 sometime early in the coming year. All members of the Society are urged to constitute themselves volunteer agents for the sale of these Classics. A considerable sum of money may be conserved if each member of the Society will undertake to solicit subscriptions to these Classics amongst his friends and colleagues, collect the money, send the order to me, and assume the responsibility of distributing the copies upon their receipt. All such orders for these Classics should be accompanied by money order, and a list of the subscribers with their addresses, in order that we may canvass them later for future issues of these Classics.

The editor and manager solicit suggestions for future Classics in this series. Let us know what you would like to have translated. We shall give your suggestions careful consideration.

Respectfully submitted,

H. H. WHETZEL, *Business Manager,*
Phytopathological Classics.

REPORT OF THE ADVERTISING MANAGER

During 1933, a total of 125 advertisements were run in PHYTOPATHOLOGY. These 125 advertisements consisted of a total of 85½ pages. Of the total advertising, 62 advertisements were nonrevenue, including exchange ads with other journals and such Society helps as Phytopathological Classics, back-number sale, meeting announcements, etc.

A study of the 1932 and 1933 advertising records shows the following facts: The income from ads dropped from $713.05 in 1932 to $561.50 in 1933, or a drop of 21.2 per cent, and the number of pages of revenue ads dropped from 44⅛ to 36½, or a 17.3 per cent drop. The number of revenue advertisers increased from 15 in 1932 to 16 in 1933, and the number of revenue advertisements increased from 61 to 63 during the same period. The number of one-page ads during these 2 years decreased from 36 in

1932 to 21 in 1933, while the number of one-half- and one-quarter-page ads increased from 25 in 1932 to 42 in 1933. From these comparisons, it appears that advertising in PHYTOPATHOLOGY is growing.

Respectfully submitted,

R. S. KIRBY, *Advertising Manager.*

COMMITTEE ON EDITING PHYTOPATHOLOGICAL ABSTRACTS

The number of abstracts submitted this year was the smallest in the last decade. The Committee deemed it unwise to impose restrictions that would curtail the program still further; accordingly, some liberties with the traditional limitations on the number of contributions acceptable from one group of authors were permitted. It appears that most of the Society membership fully took to heart the restriction against submission of abstracts that were not even intended to be presented at the meetings, but such self-denial was not universal. This tendency to use our abstract series as an outlet for almost immediate publication, and the tendency also to repeat material in the abstracts that has already been, or is soon to be, submitted (in more extended form) for publication—often indeed in PHYTOPATHOLOGY—are the most pernicious features of the present policy of the Society on abstracts. It is manifestly impossible for the Abstract Committee to know who really intends to go to the meetings and present his paper in person, or at least to have a colleague who understands the work that has been done, to substitute for him; and generally the Committee has no means of determining whether publication of the same material *in extenso* has been arranged or is contemplated. Abstinence from this sort of advantage taken of members of the Society who observe the rules, can not be enforced; it can only be requested in the spirit of fair play.

The fact that the regular monthly issues of our journal make provision for the prompt publication of brief articles and preliminary papers (when meritorious) should eliminate all grounds for using the abstracts for any other purpose than giving advance notice and leaving a permanent record of material actually presented on the programs.

The Committee has nothing further to say regarding the quality of the abstracts either as to substance or style. We recognize that they are not of uniform, and some are of very slight, merit. We think there is room for improvement, but there is likewise in the papers that PHYTOPATHOLOGY publishes in full. We regard the abstracts we accept now as not particularly inferior to those published by any other Society, even some very prominent ones. It is amusing at times to listen to the denunciation of the abstract policy of the Society, as well as of the character of the abstracts, so often proclaimed at our annual meetings, in view of the fact that probably none of us, in the eyes of someone else, invariably succeeds in compliance with the scriptural injunction: "Let him who is without sin, cast the first stone."

Respectfully submitted,

FREEMAN WEISS, *Chairman.*

REPORT OF COMMITTEE ON FOREIGN PESTS AND PLANT DISEASES

Your committee calls attention to the suggestions made last year that visitors to foreign countries be asked to make reports on foreign pests and diseases, and is pleased to report that W. C. Snyder of California has volunteered to assist in special problems such as potato wart, Colchicum smut, mosaic on Cymbidium, and the Septoria on azalea during sojourn in Europe. We hope that other members who are contemplating foreign travel may undertake similar service.

While the technical publications of foreign countries are readily available and contain much valuable information, there are many features relating to foreign pests

that are especially valuable to our regulatory service but that are not fully covered in these publications. Information of this sort might be obtained most readily by means of such reports as are commonly made on commercial subjects by the Consular Service. It is recommended that the president of the Society make inquiry through the Department of Agriculture as to whether arrangements for obtaining such information can be made.

As a matter of keeping the members of the Society informed on outstanding developments in the quarantine field, it is recommended that the U. S. Department of Agriculture be requested to present annually to the Society a report on the important foreign-pest problems that might be of interest to this country.

After a thorough discussion in this committee with members of the Committee on Quarantine and Regulatory Work regarding the desirability of merging the duties of both committees, it is recommended that this step be taken.

C. R. ORTON, *Chairman*,
W. A. MCCUBBIN,
F. D. FROMME.

REPORT OF COMMITTEE ON QUARANTINE AND REGULATORY WORK

Your committee notes as a feature of considerable significance an increasing interest throughout the world in quarantine matters as reflected in the growing number of regulatory measures recently put into effect in various countries, together with a noticeable tendency to apply more stringent types of restrictions on foreign imports. Several European nations have thus made outstanding advances in their plant-protection systems. Among the most notable recent events in this connection is the establishmet of a quarantine and regulatory service in the Republic of China. It may be of interest to members of the Society to note that among other sources of information the U. S. Bureau of Plant Quarantine now contributes a series of circulars summarizing the foreign quarantine regulations imposed by most of the countries of the world.

Among the outstanding recent outbreaks of pests in various countries, which appear to have stirred up considerable apprehension and have exerted a general stimulatory effect toward a tightening up of import restrictions, the following few cases may be mentioned:

A recent outbreak of San José scale in the Balkans is thought to have come from America *via* a European country that has been rather lax in its protective measures.

A spread of potato wart into Switzerland and Portugal is noted and there is now a record of its occurrence in Bolivia. In the case of Switzerland the infection was traced to supposedly immune seed potatoes brought from East Prussia.

There has been considerable spread of the Colorado potato beetle in France, and the alarm of other European countries concerning this pest has resulted in drastic quarantine restrictions against French potatoes. A few of these beetles were recently found in the vicinity of an English port.

A rapid and practically uninterrupted spread of the Dutch elm disease has taken place over Europe and into this country. The strong suspicion that this destructive disease originated in some eastern land and came to Europe in some such fashion as the chestnut blight was brought to America is significant from the quarantine point of view.

Some concern in Europe concerning the disease of firs due to *Rhabdocline pseudotsugae* is finding expression in measures to keep the malady from spreading to continental areas.

Examples frequently quoted to illustrate the damage that may arise from introducing a foreign species into a new habitat are the rabbit and cactus in Australia. The behavior of the muskrat recently let loose in England promises to add another to the well known list of horrible examples.

A recent conference in Pennsylvania on the potato-wart situation brings out a possibility that the limited infections of this disease in Pennsylvania, West Virginia, and Maryland may be effectively eliminated by eradication measures involving soil treatment with ammonium sulfocyanate. In connection with such a program your committee believes that eradication effort should be accompanied by extensive surveys of areas in all 3 States where wart is likely to occur.

Your committee has received a copy of the report of the special quarantine committee appointed at the summer meeting at Chicago and concurs in the views expressed in this report.

In accordance with the recommendation in the special committee's report urging concerted study in various countries of the plant pathological basis of quarantine problems, your committee believes that this society might well indicate to the officials in charge of arranging the coming International Botanical Congress in Amsterdam in 1935 our hope that some consideration of this subject may be included in the agenda. In this connection your committee believes that the relations of virus troubles, nematodes, and bacterial diseases to quarantine activities in general, are much in need of attention.

The question of merging the duties of this committee with those of the committee on Foreign Pests and Diseases has been carefully considerd by members of both committees and it is recommended that this action be taken.

C. R. ORTON, *Chairman*,
J. S. BOYCE,
H. T. GÜSSOW,
J. F. ADAMS,
W. A. MCCUBBIN.

In accordance with the foregoing recommendations the Council has named the following as a committee on Foreign Plant Diseases: C. R. Orton, *Chairman*, H. T. Güssow, J. S. Boyce, W. A. McCubbin, R. D. Rands.

COMMITTEE ON EXTENSION WORK IN PLANT PATHOLOGY

The extension committee sponsored a barely-disease conference at Dubuque, Iowa, in September that was attended by pathologists, agronomists, and commercial interests of the surrounding States aided by representatives of the U. S. Department of Agriculture extension service, grain grading service, and cereal office. As a result of this conference barley grading schools are being arranged for Minnesota, Iowa, Wisconsin, and Illinois. Barley scab and Helminthosporium head blight were the diseases given major attention.

Arrangements have been made for the Boston meeting with Dr. Chupp as chairman. Topics for discussion include: (1) New seed disinfectants and fungicides; (2) commercial seed treatment, its advantages and limitations; (3) seed treatment and other service work in the countries; (4) emergency-garden relief work; (5) new points on illustrative material and its use.

The stencil circular "The Extension Pathologist" has been issued at irregular intervals under the direction of R. J. Haskell, Extension Service and Bureau of Plant Industry, cooperating. This circular has been helpful in the exchange of ideas, and will be continued.

Respectfully submitted,

R. E. VAUGHAN, *Chairman*.

COMMITTEE ON NECROLOGY

During the calendar year 1933, there have been two deaths, as follows:

Mr. Arthur Bliss Seymour, March 29, 1933.

Dr. James Abraham Faris, September 24, 1933.

It has been learned recently also that Mr. Harold Peirce died April 12, 1932.

A. G. JOHNSON, *Chairman*,
G. P. CLINTON,
M. B. WAITE.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Committee on Elections. The committee on elections, after having opened sealed-ballot envelopes and counting ballots for each nominee, reports election of the following officers: President, N. E. Stevens, Vice-President, G. W. Keitt, Councilor, F. D. Heald.

A. N. BROOKS, *Chairman*,
H. S. CUNNINGHAM.

Auditing Committee. The committee appointed to audit the accounts of the Secretary-Treasurer and Business Manager of PHYTOPATHOLOGY has examined the books of this official and found them correct and in good order.

The committee commends the care with which these several accounts have been kept.

JOHN STEVENSON, *Chairman*,
JOHN W. ROBERTS.

Report of Temporary Committee on Plant Quarantines Appointed at Chicago Meeting 1933. The temporary committee on plant quarantines appointed at the Chicago meeting respectfully submits the following report.

We unanimously approve the suggestions of Dr. O. Appel in his Chicago paper regarding plant quarantines, namely, that the biological basis of plant quarantines is an appropriate subject of consideration by plant pathologists and that concerted world-wide study of the biological factors involved is desirable.

We feel that this Society has a definite responsibility to encourage the study and discussion of plant-disease problems in relation to quarantines.

We congratulate the authors of University of California Bulletin No. 553, "The Efficacy and Economic Effects of Plant Quarantines in California," on the discussion of the questions therein contained and commend this Bulletin to the consideration of our members.

We endorse as worthy of hearty support the present policy of the U. S. Quarantine authorities in frequently reviewing at public conferences questions of quarantine policy and procedure.

We respectfully request that this report be accepted and a copy filed with the standing committee on plant quarantines and that our temporary committee be discharged.

L. R. JONES, *Acting Chairman*,
J. DUFRÉNOY,
H. T. GÜSSOW,
H. H. WHETZEL,
E. C. STAKMAN,
W. A. MCCUBBIN,
N. E. STEVENS.

The Report of Representatives on the Council of the A. A. A. S. The duties of Society Representatives on the Council of the A. A. A. S. are chiefly voting on candidates for offices and titles for Section "G" programs. We have consistently voted in such

matters in accordance with individual judgment. If members of this Society have suggestions as to desirable choices, we shall be glad to receive them.

NEIL E. STEVENS,
H. S. CUNNINGHAM.

Resolutions Committee. The American Phytopathological Society wishes to express its thanks to the American Association for the Advancement of Science for making general arrangements for these meetings. It also is indebted to Dr. Sam Prescott of the Massachusetts Institute of Technology and his associates for arranging many of the details of the meetings.

The Society wishes to express its thanks to James Biggar, supervisor of Harvard Building arrangement facilities, to Thomas Lawlor and Paul Walker, supervisors of student aides for motion-picture projectors, lantern service, preparation of rooms, and handling of exhibits.

For the very efficient and courteous service to its members during these meetings, The American Phytopathological Society wishes to express its appreciation to the management of the Westminster Hotel. Special thanks are due to Mr. Joseph McCarthy, Manager, to Herbert Penney, who managed the banquet, and to Willard C. Stimpson, Andrew Anderson, John Olson, and Thomas Garvey.

Special applause and appreciation are extended to Director Thieelavia, Acetic Acid Doran, Toast Master Aloysius P. McGinty, The Big Bad Wolf of the Tobacco Belt and to Dr. Operculate Kitrid Sparrow, members of the expedition to McGinty Archipelago, for the mirth-provoking entertainment given members of The American Phytopathological Society and their friends who attended the annual banquet of the Society.

Deep and sincere appreciation are again extended to F. C. Meier, Secretary-Treasurer of the Society and Business Manager of PHYTOPATHOLOGY, and to H. B. Humphrey, Editor in Chief of PHYTOPATHOLOGY, and his associates for their continued, efficient services and for their constant contributions of time and energy in behalf of The American Phytopathological Society.

W. H. TISDALE,
S. M. ZELLER,
ANNIE R. GRAVATT.

ACTION OF THE COUNCIL

In addition to making the appointments of the officers mentioned earlier in this report, the Council submitted the following actions, which were approved by the Society:

1. It is recommended that, as was the case in 1933, the Editor in Chief of PHYTOPATHOLOGY be allowed actual expenses up to or within $300 for secretarial and editorial assistance.

2. That the Editor in Chief of PHYTOPATHOLOGY be authorized to select the necessary associate editors for the year 1934.

3. It is recommended that the Secretary-Treasurer be authorized to contribute $15.00 to defray expenses of the Biological Smoker in Boston.

4. It is recommended that in view of the long-established custom and the advantages derived from contacts with members of other professional groups, this Society continue the practice of meeting with the American Association for the Advancement of Science.

5. It is recommended that the Secretary-Treasurer be instructed to insert a one-page advertising notice in PHYTOPATHOLOGY for the purpose of ascertaining member personnel available for employment and opportunities available for work.

OTHER BUSINESS

The reports of officers, committees, and representatives as printed above were accepted.

A motion made by I. E. Melhus that the Chair appoint a committee to place the following resolution before the appropriate officers of the Federal Government and to cooperate with them in formulating a satisfactory program was carried:

Be it Resolved, that The Phytopathological Society believes that a State and National serious hazard prevails in the impaired state of health of our tree population, which is in sore need of study as to cause, prevalence, distribution, and destructiveness, that Congressional Act—1105—73d Congress (S–598) provides for plant-disease control and for forest research, and that, to date, no adequate phytopathological program has been undertaken under this Act, and that the Society urges upon those charged with the responsibility of administering the Act to formulate such a program in cooperation with the several States.

ARTHUR BLISS SEYMOUR

JANUARY 3, 1859—MARCH 29, 1933

Arthur Bliss Seymour was graduated from the University of Illinois in 1881 with the degree of Bachelor of Science and, in 1886, he received the degree of Master of Science from the same institution.

From 1881 to 1883, and again for a period in 1884, he was botanist for the State Laboratory of Natural History for Illinois. From 1883 to 1885, except for the period in 1884 referred to above, he was private assistant to Dr. Farlow in the Cryptogamic Herbarium at Harvard University. From 1885 to 1886, he was instructor in botany at the University of Wisconsin. From 1886 to the time of his death he served as assistant in the Cryptogamic Herbarium of Harvard University.

Mr. Seymour served as editor of cryptogamic botany for a section (A–G) of the Century Dictionary in 1888, and from 1890 to the time of his death he served as chief editor for economic fungi for the same publication. He was the author or joint author of numerous publications chiefly on mycology and plant pathology, and was compiler of that stupendous and very valuable work "Host Index of the Fungi of North America." The painstaking care and persistence with which he followed up this work exemplifies his principal ideal in life, namely, "service for others." He contributed in many important ways to the fields of mycology and plant pathology.

JAMES ABRAHAM FARIS

JULY 27, 1890—SEPTEMBER 24, 1933

James Abraham Faris was graduated from the Northwest Missouri State Teachers College at Maryville in 1913. He received the degree of Bachelor of Science from the University of Missouri in 1916, his Master's degree from the University of Nebraska in 1920, and the degree of Doctor of Philosophy from Columbia University, New York City, in 1923.

During 1916–17 Dr. Faris was Professor of Botany and Biology at Stephens Junior College, Columbia, Missouri, and during 1917–18 he was Professor of Botany at the City Junior College, St. Joseph, Missouri. From 1918 to 1920, he was Pathologist in the Office of Cereal Investigations and employed as State leader in barberry eradication in Nebraska. From 1920 to 1921 he made investigations in Santo Domingo for the Dominican Government on diseases of economic crops of that country. From 1921 to 1924, he was Research Fellow of the National Research Council and the Brooklyn Botanic Garden, Brooklyn, N. Y. From 1924 to May, 1931, Dr. Faris was Chief Pathologist of the Tropical Plant Research Foundation and the Cuba Sugar Club and was stationed at Central Baraguá, Cuba, where he served also as Assistant Director of the Sugar Planters Experiment Station. On June 1, 1931, Dr. Faris was appointed Senior Pathologist in the Division of Cereal Crops and Diseases, in charge of investigations of cereal smuts, which position he held until the time of his death.

Dr. Faris made important contributions to the scientific knowledge of plant diseases and their control, particularly the smut diseases of cereal crops and the various diseases of sugar-cane. He was a man of unusual ability and integrity, combining keen, well-balanced judgment with aggressive leadership and personal charm.

PHYTOPATHOLOGY

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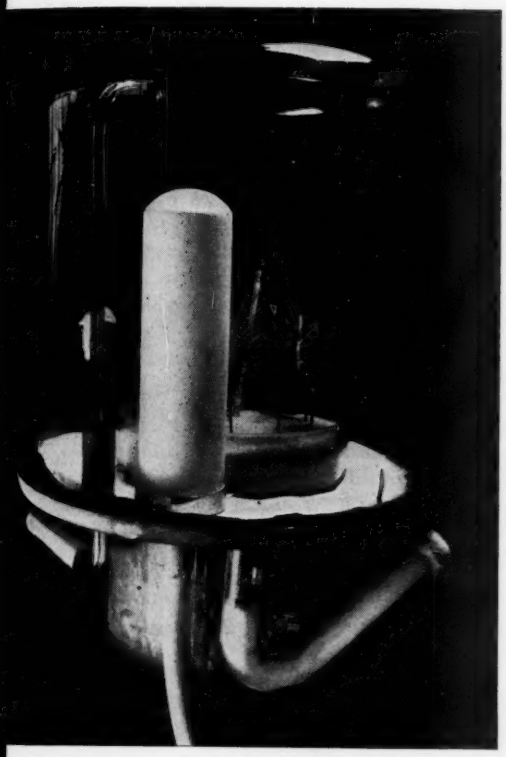

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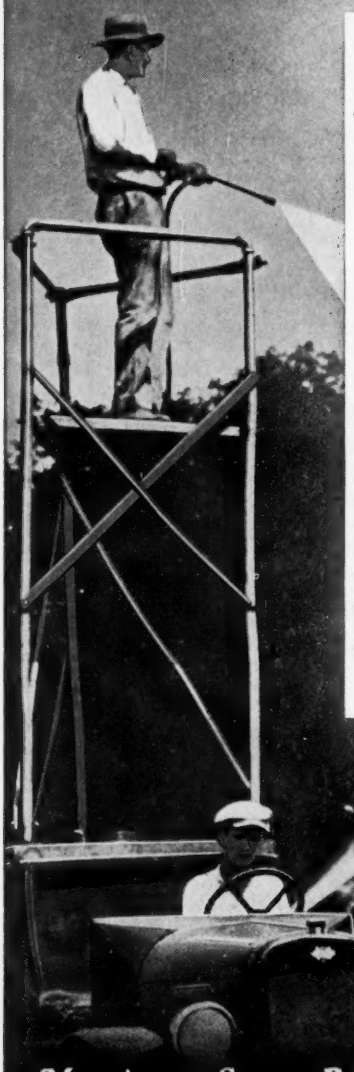

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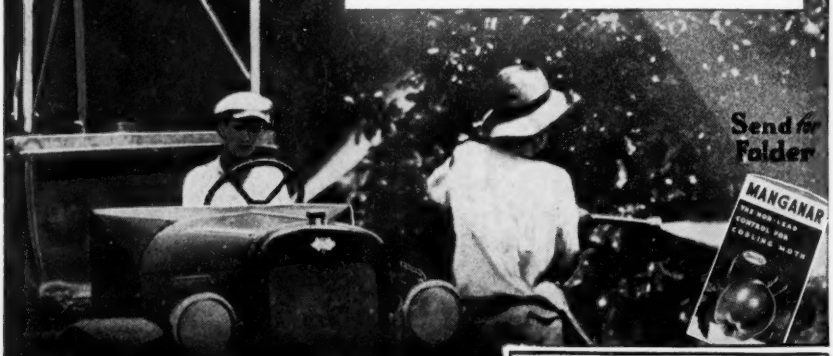

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